**Overview**

The primary aim of the Research Center for Radiation Protection is to provide a scientific basis for radiation protection and safety. Toward this goal, radiation exposure from various sources is measured, the dose-effect relationships for various endpoints are examined, and the mechanisms underlying the effects are investigated. The Research Center disseminates its research results to promote public understanding of radiation effects and to encourage the enactment of more reasonable regulations concerning the use of radiation. The scope of its activities is not limited to Japan. It has been appointed a Collaborating Centre by the International Atomic Energy Agency and the appointment was renewed this year for the next four years.

The Research Center consists of the Planning and Promotion Unit, three research programs (Radiobiology for Children’s Health Program, Radiation Risk Reduction Research Program, and Regulatory Science Research Program) and one Research and Development Team; the activities in each of these programs and the team are summarized below.

**Activities across the whole center**

Since the TEPCO Fukushima Daiichi Nuclear Power Plant (NPP) accident (March 11, 2011), the Center has organized telephone consultations which have been carried out with the help of other NIRS members. The number of consultations has reached almost 19,000.

In FY2013, the 6th International Systems Radiation Biology Workshop was held on March 5-7, 2014 as a Research Center Symposium, conjointly with DoReMi (Low Dose Research towards Multi-disciplinary Integration, supported by the EC). More than 100 researchers including 14 from foreign institutes participated in this symposium. Various research results were presented and discussed, which are related to low dose radiation effects, including studies on tumor cell proliferation and progression and mathematical analyses of the mechanism of carcinogenesis. Not only frontier research findings were presented, but also recovery operations for the Fukushima NPP accident and risk communication about radiation effects were also discussed. The symposium was very fruitful for the Center and all participating organizations.

At the close of the symposium, we invited 5 researchers for peer review on our research activities in the most recent 3 years. Prior to the review meeting, a research report was sent to each reviewer. At the review meeting, program leaders made presentations on their research activities, followed by discussion with the reviewers. A review report was immediately compiled, and reviewers finalized the review report, giving us a lot of useful suggestions and encouragement.

**Radiobiology for Children’s Health Program**

1. **Background and Objectives of the Research Program**

In this era of low birthrate and prolonged longevity in Japan, concerns about the safety of fetuses and children have been growing. Progressive increases in the use of medical radiation for children have recently forced the ICRP, IAEA and WHO to draft global initiatives on radiation protection of children.
This program carries out studies using mice and rats to provide information on the risk of cancer due to radiation exposure during fetal and childhood periods. Our studies focus on the effects of high linear energy transfer (LET) radiations i.e., neutrons and heavy ions, on fetuses and children. The ultimate objective of this research group is to propose weighting factors for both age-at-exposure and radiation quality to support the framework of radiation protection.

2. Approach to Reach the Objectives

Mice and rats were exposed to gamma rays from a $^{137}$Cs source at a dose rate of 0.6 Gy/min. Carbon ion exposure was performed using the Heavy Ion Medical Accelerator in Chiba (HIMAC) (energy, 290 MeV/u; LET, 13 keV/μm). For routine carbon ion radiotherapy, 13 keV/μm approximates the LET of the therapy beam passing through normal tissue ahead of the tumor volume. Fast neutrons (2 MeV average energy) were generated by a Be(d,n)B reaction from the Neutron Exposure Accelerator System for Biological Effect Experiments (NASBEE) and administered at a dose rate of 0.025 Gy/min.

Life shortening was investigated in B6C3F1 mice of both sexes, which are the standard strain for toxicology research. For assessment of cancer risk, various animal models for not only hematopoietic neoplasms but also solid tumors at specific sites were used.

3. Results

3.1. Lifespan shortening

Life shortening (LS) is considered as a reasonable endpoint to estimate the risk of radiation because it is representative of the total radiation effects. The results obtained are as follows:

- Female mice appeared to be more susceptible to radiation-induced lifespan shortening than male mice. The effect of gamma rays on lifespan shortening of neonate or infant mice was more pronounced than that of adult mice. Surprisingly, irradiation at the late fetal stage had little influence on lifespan shortening.

- Carbon ions were more potent in reducing lifespan than gamma rays. When acute non-cancer effects were excluded, RBE values were almost the same regardless of the age-at-exposure.

- Neutrons were extremely potent in reducing lifespan compared to gamma rays and carbon ions. The largest RBE for neutrons was observed when irradiated in utero as compared to later life stages.

3.2. Cancers

There are susceptible age windows for radiation carcinogenesis, which are organ dependent. Most organs show high radiation sensitivity in perinatal or infant stages, but the lungs and acute myeloid leukemia show susceptible age windows in the adult stage.

**Lung:** As are the results of epidemiological studies on atomic bomb survivors, irradiating Wistar rats resulted in an increase of the incidence and the hazard ratio of adenocarcinoma and squamous cell carcinoma in a dose dependent manner. The cancer risk for X-rays at infancy was lower than that at puberty and adulthood. Array CGH analysis revealed that copy number loss increased with increasing age-at-exposure. RBE values of carbon ions were 0.2, 1.3 and 2.8 for rats irradiated at 1, 3 and 7 weeks of age, respectively. The RBE values for neutrons were much higher than that for carbon ions regardless of age at exposure.

**Kidney:** Although the kidneys are one of the organs with low susceptibility to radiation carcinogenesis, the precise risks of kidney cancer after childhood exposure to radiation remain to be quantified. Perinatal exposure to radiation increased the incidence of renal cell carcinoma in Eker rats, which harbor a germ-line mutation in one of the alleles of the Tsc2 gene. But atypical tubules, which are premalignant lesions of kidney cancers, were induced at higher rates in adult rats than neonatal rats. This indicates that malignant progression is much faster when irradiated at the perinatal age than at the adult age.

The frequencies of LOH at the wild-type allele of Tsc2 were 38%, 29% and 60% in the unirradiated group and the group irradiated at 19 dpc and post-natal day 5, respectively. The pattern of LOH on chromosome 10 indicated that an interstitial-type LOH at the Tsc2 locus was only observed in radiation-induced renal tumors. Sequence analysis of Tsc2 in the LOH-negative tumors showed frame-shift mutations, deletion mutations and a missense mutation of Tsc2. Altogether, about 90% of the spontaneous tumors and 60% of the radiation-induced tumors showed inactivation of wild-type Tsc2.

**Radiation Risk Reduction Research Program**

1. Background and Objectives of the Research Program

Cancer susceptibility after exposure to radiation differs depending on individuals. Age and gender are known factors causing individual differences in radiation sensitivity. Smoking has also been identified to elevate the individual sensitivity to α-particles. In addition to these well-defined factors, there are other potential factors which have been suggested to modulate radiation sensitivity of individuals. First, variable efficiency of DNA repair resulting from single nucleotide polymorphisms (SNPs) located in genes for DNA repair-related proteins is thought to cause individual differences in radiation sensitivity. Second, there is evidence suggesting that individual radiation sensitivity is modulated by lifestyle; especially the calorie intake has been shown to have a significant impact on radiation carcinogenesis in laboratory animals.

The purpose of this program is to identify factors, whatever genetic and epigenetic, causing individual differences in radiation sensitivity, and also to present a possible way to reduce individual radiation risks by artificially regulating these factors.

2. Approach to Reach the Objectives

We consider that finding SNPs in the human population that impair DNA repair factors is critical to identify high risk groups and to establish a finely-tuned justification for radiation protection of these individuals. In the repair of DNA double strand breaks (DSBs) in animal cells, multiple protein factors are recruited and accumulated at the DSB site. If some factors have amino acid substitutions or deletions produced by SNPs which cause inability to accumulate at the DSB site, such factors could be considered no longer functional, and the cells would be hypersensitive to radiation. In order to identify such SNPs, we established a micro-
3. Research Results

We first investigated Ku70 which plays an important role in non-homologous end-joining (NHEJ) which is a major DNA repair mechanism of mammals. Using a Ku70-deficient epithelial cell line, we could show that the ectopically-expressed EGFP-tagged Ku70 accumulated at DSBs immediately when DSBs were produced by micro-irradiation with the laser. A series of deletion mutants of EGFP-Ku70 was constructed, and we could identify several domains, including the N-terminal, DNA-binding and Ku80-binding domains, deletion of which caused inability of the factor to accumulate at the DSB. In addition, we could show that a single mutation at leucine 385 abolished the ability to accumulate at DSBs. This result indicated that SNPs associated with a loss of leucine at 385 are candidate biomarkers of radiation sensitivity.

We further analyzed a second DNA repair factor Rad52 which plays an essential role in another DNA repair mechanism, homologous recombination. We observed that deletion of the eight amino acids of the C-terminal end completely abolished the recruitment of Rad52 to DSBs. Importantly, it was previously reported that three human SNPs bring about deletions of the C-terminal end of Rad52, suggesting that these SNPs are candidate biomarkers of radiation sensitivity.

Radiation-induced DNA damages are repaired mainly by NHEJ in mammalian cells, but this is not a mechanism with high fidelity. Then we examined whether individual sensitivity to stochastic effects of radiation may be reduced by artificial inhibition of NHEJ pathways in cases that it is planned to expose an individual to low level radiation. In order to explore the feasibility of this idea, we analyzed the frequency of radiation-induced mutation at HPRT gene locus using various cell lines deficient in NHEJ-related genes. As a result, mutation frequencies in these cells were found to be lower than that in their parental cells. This observation is considered to be the result of an enhanced cell killing. However, it may be considered that the radiation-protective strategy by inhibition of NHEJ pathways may be beneficial in the case where deterministic effects caused by a critically large number of cell death events are negligible.

4. Significance and Relevance

The impact of various factors on individual radiation sensitivity is currently being investigated in Europe and the US to provide a scientific basis for protection of high risk groups from radiation. However, it is important to note that some factors such as lifestyles and genetic polymorphisms are different between races and/or cultures, so that the research on these factors should be promoted in Japan separately. Thus, our research program had great significance and relevance. As well, we are planning to establish possible ways to reduce individual radiation risks by artificially regulating these factors. This strategy may be referred to as “active radiation protection” showing a possible style for next generation radiation protection.
communication. A tool for risk assessment using the latest risk information was developed to estimate radiation risk among a specified group of exposed people. The contents of the telephone consultations opened at NIRS after the Fukushima NPP accident were analyzed.

3.3. Measures for environmental protection

To develop new standards for environmental protection against radiation exposure, we carried out analysis of the transfer of radioactive materials to animals using a dynamic model. The results of the assessment showed higher activity concentration in a standard animal than that estimated by a model using a static value for the transfer factor.

3.4. Activities other than research

As activities other than research, members of the program have handled considerable numbers of requests from regulatory bodies responsible for radiation protection of the public against exposure to radioactive materials due to the Fukushima NPP accident. Members of the program also have cooperated in activities of international organizations, especially UNSCER.

R&D Team for Biospheric Assessment for Waste Disposal

The aim of the team’s current project is to provide environmental transfer parameters for radiation dose assessments from radionuclides released from radioactive waste disposal sites. To obtain suitable parameters for the Japanese biosphere, this team has been carrying out three tasks: (1) Constructing the database of environmental transfer parameters (TFs and Kds) considering climate change; (2) estimating the effects on microbial activities for the transfer parameters of $^{14}C$ in soil-plant systems; and (3) collecting the environmental transfer parameters of important radionuclides (Pu, Am, Th and Cl) by ultra-high sensitivity analysis. Some details of these are given below.

1. Constructing the database of environmental transfer parameters (TFs and Kds) considering climate change

A literature survey was conducted to estimate the effect of warm and cool temperatures on soil-to-plant transfer factors (TFs) compared to those for temperate areas. According to the IAEA Technical Report Series No.472 (TR-472), TF of Cs increased with decreasing temperature. A literature survey of Japanese TFs of brown rice showed this trend as well. Additionally, 20 samples of leafy vegetables and associated soils were collected in the southern part of Japan this year to compare the TF change with leafy vegetables grown under warm conditions. The concentrations of 20 elements in the vegetables and soils were determined and were compared with our previous data. Although there were no differences between the new data and our previous data in soils, the concentrations of Si, Co, Ni, Cd and Cs in vegetables showed differences which were more than a factor of two. More detailed study is needed; more elemental concentration data at major-to-trace levels will be added next year to provide more information.

The effective half-lives of radio-Cs in fruit trees and the concentration ratios of Cs-137 of wild animals and fishes in Fukushima Prefecture were collected to understand the behavior of Cs in the environments under the non-equilibrium condition to get apparent equilibrium constant values in transition.

2. Estimation of the effects on microbial activities for the transfer parameters of $^{14}C$

It has been recognized that $^{14}C$ (T1/2: 5,730 years) is one of the dominant radionuclides affecting exposure dose from TRU wastes. The measurement method for gaseous chemical species containing $^{14}C$ released from soil was investigated. A new method to quantify the gasification of $^{14}C$ from soil was also studied, focusing on microbial activity. In our previous study, it was revealed that soil microorganisms strongly affected the chemical form changes of $^{14}C$ in low molecular weight organic carbon compounds. Thus respiration activity of soil microorganisms was measured using the INT Formazan reaction.

Although individual mechanisms of $^{14}C$ changes with time were studied, it is necessary to connect laboratory study results and field observations. Thus construction of a realistic $^{14}C$ model is necessary, but in our previous model, the gasification process had a single parameter though it should be affected by both temperature and CO2 concentration in the air. A literature survey was conducted to obtain default parameter data on uptake of carbon by rice plants from the ambient air. Then the model was updated and sensitivity tests were carried out to see the applicability of the newly included parameter values.

3. Collecting the environmental transfer parameters of important radionuclides (Pu, Am, Th and Cl) by ultra-high sensitivity analysis

This year, our study focused on the development of highly sensitive analytical methods for the determination of Pu isotopes and Cl in soil samples. For Pu analysis, we compared the sensitivities of two sector-field ICP-MS instruments, the Element 2 and the newly introduced Element XR. Due to the newly developed jet-interface in the Element XR, a factor of 10 sensitivity improvement is achieved compared with the Element 2. Combined with a high-efficiency samples introduction system (Aridus II), a sensitivity of 100 M cps/ppb U or Pu was obtained, which provides the required sensitivity for Pu isotope analysis of rice samples which we expect to conduct next year. To determine Pu isotopes in soil samples, we developed a two-stage anion-exchange chromatographic separation procedure, in which extremely high U decontamination factor of $2x10^5$ was achieved. The troublesome UH+ interference was completely eliminated. An analytical method for accurate determination of Pu isotopes in soil samples was established, and applied to the determination of $^{239,240}$Pu and $^{238,240}$Pu in school ground soil samples collected in the 1970s to obtain the background data on Pu contamination in school ground soils in central-east Japan. For soil Cl analysis, two methods, water extraction-IC measurement and TMAH extraction-ICP-MS analysis, were evaluated. The obtained results showed that for samples with Cl concentration higher than 100 mg/kg, good agreement between these methods was obtained, while for the samples with Cl concentrations lower than 100 mg/kg, the concentration of Cl obtained with the TMAH-ICP-MS analysis method was higher than that of the water extraction-IC measurement method. Further work is planned for next year to complete establishment of the sensitive Cl analytical method.
Potting mix is often composed of a variety of soils such as akadama-tsuchi, kuro-tsuchi, and so on. In this study, root uptake of radiocesium ($^{134}\text{Cs}$, $^{137}\text{Cs}$) was evaluated for mini cabbage plants grown on potting mixes with different concentrations of radiocesium. Three potting mixes were prepared that contained kuro-tsuchi and akadama-tsuchi mixed with one other radiocesium contaminated material: leaf-mold (at two radiocesium concentrations) or sludge. (Akadama-tsuchi was contaminated with radiocesium, but radiocesium in kuro-tsuchi was below the detection limit.) Mini cabbage plants were grown using the three mixtures. At the harvest date, the concentrations of radiocesium in the mini cabbage edible plant parts were below the new standard limit for general foods in Japan, that is, 100 Bq kg$^{-1}$ wet. The highest soil-to-plant transfer factor of 0.69 (dry weight basis) was observed for the potting mix composed of contaminated akadama-tsuchi, kuro-tsuchi, and slightly contaminated leaf-mold. Akadama-tsuchi is the most commonly used material for potting mixes, and thus it may be necessary to pay attention to the radiocesium contamination in akadama-tsuchi.

### Introduction

The Fukushima Daiichi Nuclear Power Plant (FDNPP) was severely damaged by events that followed the magnitude 9.0 earthquake and the subsequent huge tsunami of March 11, 2011. Radionuclides, which were released because of the plant damage, have contaminated the environment, and among the radionuclides deposited on the surface soil, relatively long-lived radionuclides, such as radiocesium ($^{134}\text{Cs}$, $^{137}\text{Cs}$), would be taken up by agricultural crops. Intake of agricultural crops contaminated with radionuclides causes internal radiation exposure.

The safety of most agricultural crops has been ensured by a new standard limit for radionuclides in foods, which was enforced by the Japanese government on April 1, 2012. However, measurement of radiocesium in crops, which were harvested from home gardens, has not been carried out before their consumption in many cases. To ensure safety and security, it is important to obtain a better understanding of transfer of radiocesium to crops from the ingredients of commercial potting mixes.

### Materials and methods

Akadama-tsuchi, kuro-tsuchi, and two kinds of leaf-mold (slightly contaminated leaf-mold and heavily contaminated leaf-mold) were purchased from a gardening center in 2011. Sludge was obtained from a drinking water treatment plant in Saitama Prefecture. The concentrations of radiocesium in each ingredient are listed in Table 1. The presence of $^{134}\text{Cs}$ in these ingredients (except for kuro-tsuchi which was below the detection limit) suggests contamination from radiocesium released by the FDNPP accident.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>$^{134}\text{Cs}$ (Bq/kg-dry)</th>
<th>$^{137}\text{Cs}$ (Bq/kg-dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akadama-tsuchi</td>
<td>$1.6 \times 10^1$</td>
<td>$2.4 \times 10^1$</td>
</tr>
<tr>
<td>Kuro-tsuchi</td>
<td>DL*</td>
<td>$4.5 \times 10^0$</td>
</tr>
<tr>
<td>Slightly contaminated leaf-mold</td>
<td>$5.5 \times 10^0$</td>
<td>$5.1 \times 10^0$</td>
</tr>
<tr>
<td>Heavily contaminated leaf-mold</td>
<td>$9.9 \times 10^0$</td>
<td>$1.5 \times 10^0$</td>
</tr>
<tr>
<td>Sludge</td>
<td>$6.7 \times 10^0$</td>
<td>$1.0 \times 10^0$</td>
</tr>
</tbody>
</table>

* Detection limit: less than 2.7 $\times 10^0$ Bq/kg -dry
Three kinds of potting mixes were prepared by using these materials. First, the same volumes of akadama-tsuchi and kuro-tsuchi (1:1) were mixed well (base soil). Thirty grams chemical fertilizer (8% nitrogen, 8% phosphorus, and 8% potassium by weight) was mixed into 8-L base soil. To grow the mini cabbage plants, a certain amount of the slightly contaminated leaf-mold (300 mL), the heavily contaminated leaf-mold (200-mL dry), or sludge (300-mL) was mixed into 500-mL base soil containing chemical fertilizer. These mixes were identified as potting mixes No.1, 2, and 3, respectively.

Two pots were filled with each of the three mixes and one seedling of mini cabbage (Brassica oleracea Capitata) was planted in each pot. The plants were grown for 79 days under natural light. The temperature was controlled at 25 ± 5°C during the cultivation period. Mini cabbages were harvested on June 27, 2012 and divided into edible and core parts. These parts and potting mixes were separately dried and powdered using a grinder for the analysis of radiocesium.

The concentration of radiocesium in the samples was quantified using a germanium detecting system (Seiko EG&G). The measurement values were corrected to harvest date.

The soil-to-plant transfer factors (TFs) were calculated as the ratio of the radiocesium concentration in the edible part of mini cabbage (Bq kg⁻¹ dry weight) to its concentration in potting mix (Bq kg⁻¹ dry weight)

Table 2 Radiocesium concentrations of the samples and TF

<table>
<thead>
<tr>
<th>Number of potting mix</th>
<th>Ingredient*</th>
<th>Concentration (Bq kg⁻¹-dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Potting mix</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¹³⁷Cs</td>
</tr>
<tr>
<td>1 Slightly contaminated leaf-mold</td>
<td>1.3.E+01</td>
<td>2.1.E+01</td>
</tr>
<tr>
<td>2 Heavily contaminated leaf-mold</td>
<td>9.6.E+02</td>
<td>1.5.E+03</td>
</tr>
<tr>
<td>3 sludge</td>
<td>1.4.E+03</td>
<td>2.1.E+03</td>
</tr>
</tbody>
</table>

*All potting mixes contained Akadama-tsuchi, Kuro-tsuchi, and chemical fertilizer. **TF: the ratio of ¹³⁷Cs in the edible part of mini cabbage (Bq kg⁻¹-dry) to its concentration in potting mix (Bq kg⁻¹-dry).

Results and discussion

The radiocesium concentrations of the samples at the harvest date are summarized in Table 2. Radiocesium was detected in edible and core parts which suggested that root uptake of radiocesium occurred for this vegetable. It was found that the plant samples obtained from the plants grown in the potting mix with the highest radiocesium concentration, did not have the highest amount of radiocesium. Potting mix No.3 had the highest radiocesium concentration of 3.5 × 10³ Bq kg⁻¹, while the highest radiocesium concentration in the edible and core parts of the plant were observed with the potting mix No.2. These results suggest that the transfer of radiocesium from soil to plants is affected by the ingredients for potting mix.

The radiocesium concentrations were compared between edible and core parts (Table 2), and a significant difference (t-test, P <0.05) was found for the mini cabbage grown in the potting mix No.1. Removal of the core parts and not eating them may reduce the radiocesium ingestion. On the other hand, Ban-nai et al.[2] showed that there were no differences in the concentration of ¹³⁷Cs between edible and other parts of cabbage, and thus more studies on the distribution of radiocesium in crops is necessary.

To compare the transfer of ¹³⁷Cs among the different potting mix samples, TF values were calculated using the concentration of ¹³⁷Cs in edible parts and the potting mixes (Table 2). The values varied among the samples, and the highest value was obtained for the mini cabbage harvested from the potting mix No.1. The main source of ¹³⁷Cs for the potting mix No.1 was akadama-tsuchi. Probably, ¹³⁷Cs in akadama-tsuchi would be more readily available to mini cabbage compared to that in leaf-mold and sludge. Akadama-tsuchi is inorganic volcanic soil and has minute holes allowing water retention. These holes may be responsible for the high TF value. Since akadama-tsuchi is the most commonly used soil in commercial potting mixes in Japan, attention should be paid to the radiocesium contamination of akadama-tsuchi.

Tsukada and Hasegawa [3] reported that the geometric mean of TF values of ¹³⁷Cs for cabbage which was harvested in Aomori Prefecture during the early 1990s was 0.026 and that its 95% confidence interval was from 0.0021 to 0.33. These contaminations of ¹³⁷Cs resulted from the atmospheric nuclear weapon tests in the 1960s and the Chernobyl accident in 1986. Therefore, it would appear that it is relatively hard to transfer ¹³⁷Cs from soil to cabbage by an aging effect. As expected, the TF value for the potting mix No.1 sample exceeded the 95% confidence range, and the TF values for the potting mixes No. 2 and No. 3 were higher than the geometric mean of the previously reported TF values [3]. TF values in this study would decrease with time.

The Japanese government has enforced the new standard limit for radionuclides in general foods (100 Bq kg⁻¹). This standard is based on the concentration on fresh weight basis. Radiocesium concentrations reported here were on dry weight basis, therefore, the values were converted to the concentrations on fresh weight basis. The radiocesium concentrations in edible parts were 4.9 Bq kg⁻¹ for the potting mix No.1, 65.1 Bq kg⁻¹ for the potting mix No.2, and 61.9 Bq kg⁻¹ for the potting mix No.3. In each crop part sample, the concentrations of radiocesium were below 100 Bq kg⁻¹ of fresh weight.

References

[2] Ban-Nai T, Muramatsu Y, Yanagisawa K: Transfer factor of some selected radionuclides (radioactive Cs, Sr, Mn, Co and Zn) from soil to leaf vegeta-
A large amount of radiocesium was discharged in the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident of March 2011. This highlight describes the use of open-source food monitoring data to evaluate ecological half-lives (Teco) of radiocesium in some marine biota caught offshore of Fukushima Prefecture. Data were collected in two regions (north and south) with respect to the FDNPP site to obtain the regional $^{137}\text{Cs}$ concentration decreasing trend and then, the estimated Teco in marine biota were obtained. Teco values were then compared with biological half-life (Tb) estimated in laboratory settings. The ratios of Teco to Tb were inconsistent among different groups of marine species. The Teco / Tb ratios for brown seaweeds and bivalves were each approximately 1, however for demersal fish ratios ranged from 4.3 to 15. The reasons for the different ratios of Teco to Tb may be attributed to environmental and ecological factors, such as different trophic levels and metabolic changes.

**Definition of ecological half-life**

The time required for the biological elimination of 50% of a radionuclide from a living plant or animal body is called the biological half-life (Tb). When the radionuclide is actually taken into a living body, both physical decay and biological elimination occur together; therefore, the term ‘effective half-life (Teff)’, is used which is expressed as $1/T_{eff}=1/T_p+1/T_b$. However, in a natural environment, when radioactive contamination occurs, it is important to investigate how long the radionuclide persists in a population of a certain species, because the rate is influenced by ecological factors, including both abiotic and biotic factors. Ecological niche affects the radionuclide fate in a food web for example. For such cases, the term ‘ecological half-life’ (Teco) has been used. In this study, the term Teco is used to describe the time required for a 50% decline of a radionuclide in a population in a natural ecosystem. Teco typically has short-term and long-term components. The first component likely reflects the biological elimination rate of the radionuclide, while the second component likely reflects the biogeochemical cycles of the radionuclide in the ecosystem. Thus, eventually, the radionuclide concentration reaches a dynamic equilibrium between organisms and the ecosystem, and also decreases as a result of physical decay. However, in this study, only

![Fig.1 Time trend of $^{137}\text{Cs}$ concentrations in edible part of marine species collected offshore of Fukushima Prefecture (Bq/kg-wet) after March 11, 2011.](image)

(A) Arame, northern sea urchin and Sakhalin surf clam in the southern region and (B) greeling, and (C) Japanese common skate in both regions.
the short-term component was calculated because the data collection period was not long enough to estimate the long-term component. Thus, $T_{\text{eco}}$ is estimated by $\ln 2 \cdot \lambda_{\text{eco}}^{-1}$, where $\lambda_{\text{eco}}$ is the $^{137}$Cs ecological loss rate in a population and it is obtained from the slope of the exponential decline in $^{137}$Cs concentration in the population over time.

Data sources
Open-source $^{137}$Cs concentration data for edible marine biota caught offshore of Fukushima Prefecture together with the sample collection date were taken from monthly food monitoring reports provided on the website of the Ministry of Health, Labour and Welfare of Japan (http://www.mhlw.go.jp/shinsai_jouhou/shokuhin.html). $^{137}$Cs concentration data for a total of about 170 edible marine species have been reported; however, the sampling intervals were random and not consistent among different species. Thus, we selected 16 species which had data available from March 11, 2011 through the end of December 2012 (651 days), and we calculated the $T_{\text{eco}}$ values [1]. In the same manner, the results for nine species were obtained using data collected up to 800 days after March 11, 2011 and they are reported here. The data were then separated into two regions based on the relative direction of the FDNPP accident, i.e., north and south, for four fish species ranged from 0.9 to 1.8.

The $T_{\text{eco}}/T_b$ ratios are listed in Table 2. These ratios varied between the different major groupings, e.g., being about 1 for brown seaweed and bivalves, and 4.3-15 for demersal fish. After the FDNPP accident, $^{137}$Cs concentration in the surface water decreased by a factor of 1000 between April to May 2011; this prompt and continuous advection due to currents, may, to some extent, have created similar conditions to those of laboratory experiments on $T_b$. Thus $T_{\text{eco}}$s for seaweeds and shellfish results were similar to $T_b$s. Although $^{137}$Cs in most seaweeds, molluscs and crustaceans became lower than the detection limit within 800 days after March 11, 2011, $^{137}$Cs concentrations in demersal fish were still being measured, which caused higher $T_{\text{eco}}/T_b$ ratios. In the long-term under which equilibrium conditions were reached, no biomagnification would occur because the water to marine species $^{137}$Cs concentration ratios were almost the same among various species [2]. However, in the short-term after the accident, an apparent biomagnification might occur due to trophic levels and metabolic changes accompanying with growth; as the trophic level increases, prey have higher $^{137}$Cs concentration for longer time periods.

It is important to measure $T_b$ to know elimination mechanisms, however, $T_b$ cannot indicate the actual phenomena. Investigating $T_{\text{eco}}$ of various species is necessary to understand the uptake and elimination mechanisms of $^{137}$Cs in marine biota in the actual field.

Table 1 Ecological half-life ($T_{\text{eco}}$) of $^{137}$Cs for each species caught off Fukushima.

<table>
<thead>
<tr>
<th>Species, Teco</th>
<th>North</th>
<th>Species, Teco</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species, Teco</td>
<td></td>
<td>Species, Teco</td>
<td></td>
</tr>
<tr>
<td>Seaweeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arame</td>
<td>50</td>
<td>Bladderwrack</td>
<td>54</td>
</tr>
<tr>
<td>Shellfish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sakhalin surf clam</td>
<td>91</td>
<td>Scallop</td>
<td>75</td>
</tr>
<tr>
<td>Demersal fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flatfish</td>
<td>310-367</td>
<td>Flatfish</td>
<td>24 (avg.)</td>
</tr>
<tr>
<td>Marbled sole</td>
<td>281-452</td>
<td>Place</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 2 Comparison of field data ($T_{\text{eco}}$) and laboratory data ($T_b$).

<table>
<thead>
<tr>
<th>Species</th>
<th>Teco/Tb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arame</td>
<td>0.93</td>
</tr>
<tr>
<td>Bladderwrack</td>
<td>1.2</td>
</tr>
<tr>
<td>Sakhalin surf clam</td>
<td>13-15</td>
</tr>
<tr>
<td>Scallop</td>
<td>4.3-7.0</td>
</tr>
</tbody>
</table>

References
Introduction

It is well known that children are more susceptible to carcinogenic effects of radiation as compared with adults. Epidemiological studies of atomic bomb survivors, patients undergoing radiation-based medical treatments and victims of nuclear power plant accidents show that exposure at a young age results in higher risk of cancer compared with exposure during adulthood. Thus, there is a critical need to make efforts for assessing risk and developing prevention strategies for radiation-induced cancer after childhood exposure.

Calorie restriction (CR) has been known to increase mean lifespan by decelerating the rate of aging and inhibiting tumor formation in a variety of species. CR has been reported to decrease occurrence of not only spontaneous but also chemical carcinogen-induced tumors in rodents and non-human primates. CR has also been reported to have effects on radiation-induced leukemias [1], but data on tissues other than bone marrow are lacking. Although CR has been proposed as a strategy for preventing cancers, CR during childhood is not appropriate because of the potential malnutrition and disrupted endocrine function. A recent report using a chemical carcinogen-induced mouse skin tumor model shows that CR during the cancer promotion phase is more effective than during the tumor initiation phase [2]. Because radiation-induced carcinogenesis is thought to be a relatively long-term process, we propose that adult-onset CR during the tumor promotion/progression phase could be a valuable strategy in the prevention of tumors after childhood exposure to ionizing radiation.

In this highlight, we show that adult-onset CR is effective for suppressing late-occurring tumors in both non-irradiated and irradiated mice. However, CR was less effective for early-occurring radiogenic tumors such as thymic lymphoma (TL) and early-occurring non-TL [3].

Results

1. Effect of calorie restriction on lifespan

Changes in overall survival ratio are shown in Fig.1. Irradiation resulted in statistically significant lifespan shortening by 38%. Irrespective of irradiation, CR significantly extended the overall lifespan of the non-irradiated and 3.8 Gy irradiated groups by 19.6% and 19.2%, respectively. The lack of a difference between the lifespan extension between non-irradiated and 3.8 Gy irradiated groups indicates that CR contributes to lifespan extension through mechanisms that are independent of radiation effects.

2. Effect of calorie restriction on cancer prevalence

In this study, several types of cancers were detected. TL and early-occurring non-TL, which were the main causes of early death, were seen only in the irradiated groups. After one year, late-occurring non-TL, liver, and lung tumors were detected in both non-irradiated and irradiated groups. Specifically, the incidence of TL in the 3.8 Gy-95 kcal group was 20.0%, and the incidence in the 3.8 Gy-65 kcal group was 14.3%.
CR appeared to improve TL-free survival although the difference was not statistically significant.

Lymphomas that originated from lymphoid tissues other than the thymus, such as the lymph nodes or spleen (collectively called here non-TL), appeared to be classified into two types with respect to tumor development time: early and late. The two types were separated by the onset times and the time lag for these two lymphomas was >200 days. We analyzed early- and late-occurring non-TL separately. The incidence of early-occurring non-TL in the irradiated groups on both diets was ~10%. Early-occurring non-TL-free survival were not statistically affected by CR. The incidence of late-occurring non-TL in non-irradiated mice was 48.3% in the 95 kcal group and 20% in the 65 kcal group. The incidence of late-occurring non-TL in the irradiated groups was unexpectedly reduced from 16.7% to 9.5% in the 65 kcal group. CR significantly improved the late-occurring non-TL-free survival in both the non-irradiated and irradiated groups.

The predominant liver tumor type observed was hepatocellular carcinoma (HCC). Exposure to 3.8 Gy at 1 week of age significantly increased the incidence of HCC from 13.3% to 46.7% in the 95 kcal group and from 15.0% to 31.7% in the 65 kcal group, suggesting that liver tumors are radiogenic. CR significantly improved HCC-free survival in both the non-irradiated and 3.8 Gy irradiated groups.

The predominant lung tumor type was adenocarcinoma; we detected no effect of radiation on its incidence. In the 95 kcal groups, radiation did not increase adenocarcinoma incidence, while in the 65 kcal groups, adenocarcinoma incidence was even lower in the 3.8 Gy group than in the non-irradiated group. The effect of CR was evident in the 3.8 Gy groups: the incidence of lung adenocarcinoma was reduced from 25.0% in the 95 kcal group to 6.3% in the 65 kcal group. CR significantly increased the lung adenocarcinoma-free survival in both the non-irradiated and 3.8 Gy irradiated groups.

3. Risk of the various tumor types: Cox proportional hazard analysis

Multivariate Cox proportional hazard analysis was performed to assess the hazard ratio (HR) of cancer development (3.8 Gy vs. 0 Gy or 65 kcal vs. 95 kcal). Table 1 shows the HRs of cancer risk (3.8 Gy vs. 0 Gy) for various organs in the 95 kcal and 65 kcal groups. Radiation affected overall lifespan equally in the two diet groups. CR reduced HRs in a tissue-dependent manner. The tumors most affected by CR were non-TL and lung tumors followed by HCC. CR also affected overall lifespan and lifespan of tumor-free mice. The effect in the 3.8 Gy irradiated group was smaller than that in non-irradiated groups, suggesting that CR is less effective for radiation-associated diseases. This was mainly because early-occurring radiogenic TL and non-TL were not highly influenced by CR.

Summary

We showed here that irrespective of CR, 3.8 Gy of X-ray exposure shortened lifespan by 38%, and irrespective of irradiation CR extended lifespan by 20%. Adult-onset CR reduced the risk of hepatocellular carcinoma, late-occurring non-TL, and lung tumors but not TL or early-onset non-TL. The ability of CR to prevent late-occurring tumors was the same for non-irradiated and irradiated mice, indicating that the mechanism(s) by which CR influences cancer is independent of irradiation. In summary, adult-onset CR significantly inhibits late-occurring tumors in a tissue-dependent manner regardless of infant radiation exposure.

References


<table>
<thead>
<tr>
<th>Table 1 Summary of hazard ratio (HR) for major tumors</th>
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<tr>
<td>Radiation vs. 0 Gy</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Overall lifespan</td>
</tr>
<tr>
<td>Lifespan of tumor-free mice</td>
</tr>
<tr>
<td>TL</td>
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<tr>
<td>Early-occurring non-TL</td>
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<tr>
<td>Late-occurring non-TL</td>
</tr>
<tr>
<td>HCC</td>
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<tr>
<td>Lung adenocarcinoma</td>
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</tbody>
</table>

ND: not detected
Introduction

In recent years, there has been a growing concern in society about the risks associated with exposure to ionizing radiation, especially for children. With respect to radiation-induced cancer, studies of atomic bomb survivors and other cohort studies suggest that age at exposure modifies the radiation effects on cancer mortality. The relative risk of acute lymphocytic leukemia (ALL) is higher after childhood exposure than adult exposure, while the risk of acute myeloid leukemia (AML) remains constant or increases slightly increasing age at exposure. Animal studies also have shown that the incidence of radiation induction of AML is minimal when irradiation is carried out during in utero or infancy periods, but it reaches a maximum when irradiation occurs as an adult. Recent studies postulated that AML arises from hematopoietic stem cells (HSCs), suggesting that HSCs are also the target of radiation-induced AML. Despite the increase in knowledge of stem cell biology, there is still little information on the role of age in radiation effects of stem cells.

In the present study, we aimed to clarify the effect of age at exposure on HSC survival after radiation and the association of survival with the age effect on radiation-induction of AML. This work has been published in *Radiation Research* [1].

Results

Survival of CFUs after radiation exposure

Female C3H/He mice, which are prone to radiation-induced myeloid leukemia, were irradiated with various ages (1, 3, 8, and 14 weeks after birth) with $^{137}$Cs γ rays at 0, 2, 4, and 6 Gy, and the number of HSCs in bone marrow tissue was evaluated using the CFU-S assay. Also, the number of hematopoietic progenitor cells was evaluated using the in vitro CFC assay, for CFU-granulocyte macrophages (CFU-GMs) and burst-forming unit-erythroid cells (BFU-Es) and CFU-granulocyte, -erythrocyte, -monocyte, and -megakaryocyte (CFU-GEMM). The number of the CFU-S was significantly reduced in 1-week-old mice as compared with the older mice after 2 Gy exposure (Fig.1A). Also, the numbers of CFU-GM and BFU-E colonies from 1-week-old mice were significantly reduced as compared with the mice at other ages after radiation exposure (Figs. 1B, 1C). No significant changes were observed in CFU-GEMM (Fig.1D), which might be because the number of colonies was so small.

Expression of chemokine and cytokine genes

By using microarray analysis, we compared gene expression profiles of bone marrow tissues from mice sham irradiated or 2 Gy irradiated at 1 week and 8 weeks of age. Principal component analysis (PCA) indicated that gene expression profiles could be distinguished by age at exposure, and also time after exposure (Fig.2A). Pathways were differentially affected by time after exposure among different age groups, and 25 pathways including chemokine pathways were identified (Fig.2B). Then, by qPCR, we compared expression levels of several radio-protective chemokines or cytokines in bone marrow tissues between mice sham irradiated and those 2Gy irradiated at 1-week and 8-weeks of age. Among these, expressions of Csf2 and Fgf1 changed negligibly in the 1-week-old mice, while their expression in the 8-weeks-old mice increased significantly after irradiation (Figs. 2C, 2D). Also time-dependent increases in expressions of Kitl (Fig.2E) and Il1b (Fig.2F) after irradiation were observed in the 8-weeks-old mice, although the increase in Il1b expression was not statistically significant.

Summary

In this study, we demonstrated that an increased radioresistance in adult HSCs might result from the induction of radio-protective cytokines, such as Csf2, Fgf1 and Kitl, and possibly Il1b, after radiation exposure. Our findings imply that increased radio-resistance in adult HSCs may be associated with an increased risk of AML.

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Fig. 1 Effect of radiation exposure on survival of HSCs and hematopoietic progenitor cells. The numbers of colonies of CFU-S (A), CFU-GM (B), BFU-E (C) and CFU-GEMM (D) of 1, 3, 8, and 14-weeks-old mice after exposure to 0, 2, 4 and 6 Gy of γ-rays are shown. The asterisks mark differences that are statistically significant (p < 0.01).

Fig. 2 Array expression profile and radio-protective cytokine/chemokine gene expression in bone marrow tissues. PCA of bone marrow tissue samples from 1-week-old and 8-weeks-old mice after 0 h, 6 h and 24 h after 2 Gy exposure (A). Pathways affected by time after exposure among different age groups (B). qPCR analysis of radio-protective chemokine or cytokines: Csf2 (C), Fgf1 (D), Kitl (E) and Il1b (F) in bone marrow tissues. The asterisks mark differences that are statistically significant (p < 0.01).

References

Breast cancer risk factors

Worldwide, breast cancer represents 16% of all cancer incidences among women and 13.7% of cancer deaths. Although breast cancer rates have traditionally been lower in Japan than in the United States, breast cancer incidence is expected to rise significantly. It was shown that women who had received medium or high-dose ionizing radiation to the chest (for example, as treatments for other cancers, such as Hodgkin’s lymphoma) have a relative risk of breast cancer between 2.1 and 4.0. By age 45 years, up to 20% of women exposed to chest ionizing radiation for a pediatric malignancy are diagnosed with breast cancer. New data are coming to light indicating that even low dose exposures (such as diagnostic chest X-rays for tuberculosis or pneumonia) might raise this risk. Sex steroid hormones such as estrogen and progesterone play a crucial role in the development and homeostasis of the mammary gland, by regulating proliferation, differentiation and apoptosis. Evidence from the last few decades supports the idea that accumulated exposure to steroid hormones (for example in post-menopausal women under hormonal replacement therapy) is also a risk factor for breast cancer. The interplay between steroid hormones and radiation-induced risks has been described in a number of studies. For example, we have shown that progesterone protects cultured mammary cells against radiation-induced apoptosis and increases the number of proliferating cells containing chromosomal damage [1]. However, our knowledge of hormonal action in the irradiated breast is far from complete and new discoveries are challenging some established paradigms.

Cancer stem cells

Recently, a lot of attention has been given to a small population of malignant cells thought to be responsible for tumor maintenance and initiation of relapse. These cancer stem cells (CSCs) possess the ability to self-renew (thus to form tumors) and to cause the different lineage of cancer cells comprising a tumor. Breast CSCs were first observed by Al Hajj et al. [2], who described the existence of a subpopulation of CD44+CD24−ESA+ lineage human breast cancer cells capable of initiating tumors in immune-deficient NOD/SCID mice. CSC populations have been defined using several combinations of cell-surface markers, such as CD44+CD24−, or by measuring cellular activities, such as the expression of aldehyde dehydrogenase (ALDH). In a recent study, it was shown that breast cancer cell lines contain breast CSCs. CSCs may arise from normal stem cells, or from a differentiated progenitor, which acquired self-renewal abilities. CSCs are thought to be radio-resistant and have a distinct molecular signature.

Steroid hormones in the breast

Both estrogens and progesterone have strong proliferative effects on stem/progenitor cells. Several studies have shown that progesterone regulates genes (Notch pathway genes DLL-1, DLL-3, IL6, PRSS2, Interleukins IL6 and IL8 and others) is potentially involved in stem cell regulation. Estrogen was recently shown to stimulate CSC expansion through FGF signaling. It was also shown that radiation exposure or steroid hormones can contribute to the initiation of epithelial-to-mesenchymal transition (EMT) and the expansion of CSCs subpopulation. However, to date, the potential involvement of steroid hormones in the radiation-triggered EMT is unknown. New developments have also brought light onto the molecular mechanisms of hormonal action. In the normal human breast, nuclear estrogen and progesterone receptors (ER and PR, respectively) are expressed in only 15 to 30% of the luminal epithelial cells and not in other cell types. It is thought that receptor-containing cells secrete paracrine factors that influence the proliferation and activity of nearby receptor-negative cells. Recent investigations have shown that cultured MCF10A normal epithelial cells that do not express PR are nonetheless responsive to progesterone. Furthermore, CSCs can be generated during the transformation of MCF10A cells.
Ionizing radiation and steroid hormones generate cancer stem cells

In order to evaluate whether the modulation of radiosensitivity and radiation-induced breast cancer risk might involve CSCs, we measured the proportion of ALDH$^+$ and CD44+/CD24- cells in cultured breast cell lines [3]. Depending on the cell line, both exposure to ionizing radiation (X-rays) and steroid hormone treatment (progesterone and estrogen) could stimulate the expansion of CSCs. These CSCs exhibited higher tumorsphere-forming abilities and increased radioresistance. In irradiated MCF10A cells, progesterone action was independent of PR expression.

In MCF10A cells, progesterone triggers oxidative stress as well as cancer- and stemness-associated miRNA regulations

The stimulation of reactive oxygen species levels by progesterone in MCF10A cells is consistent with earlier reports showing increased mitochondrial activity (observed as increased mitochondrial potential) and the subsequent inhibition of Fas-induced apoptosis. In addition to non-genomic effects, progesterone directly triggered miRNA regulations (such as the downregulation of miR-22-3p and miR-29c-3p and the upregulation of miR-328 and miR-98-5p) consistent with cancer-related processes and stemness (Fig.2).

Conclusion

Progesterone directly triggered miRNA regulations and modulated the radiosensitivity of normal breast epithelial cells lacking the expression of PR, suggesting that the classical model of hormonal paracrine action in the normal breast may need to be completed. Furthermore, the combination of progesterone treatment and radiation exposure was capable of generating CSCs and might trigger or contribute to cancer initiation events.

Our results suggest that progesterone might influence radiation-induced breast cancer risk by generating tumor-initiating breast CSCs. In order to decrease the potential risks of breast cancer resulting from chest ionizing radiation exposure, it might be useful to take into account the variability of progesterone levels during the menstrual cycle and between individuals. Our results also shed additional light on elevated breast cancer risks in women treated with hormone replacement therapy. Further investigations are needed to better understand the mechanisms involved in PR-independent progesterone action in the normal breast and the generation of CSCs after exposure to ionizing radiation, in particular in the low-dose range.

References

Most hydrogen peroxide-induced histone H2AX phosphorylation is mediated by ATR and is not dependent on DNA double-strand breaks

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DNA double-strand breaks (DSBs) are the most serious damage to cells exposed to ionizing radiation (IR). The nuclear foci of phosphorylated histone H2AX ($\gamma$H2AX) are frequently used as a marker to monitor induction and repair of DNA double-strand breaks (DSBs) following exposure to IR or other DNA damaging agents. However, our recent study showed that $\gamma$H2AX foci induced by oxidative stress in hydrogen peroxide (H$_2$O$_2$)-treated cells are not associated with DSBs. DSBs and $\gamma$H2AX

H2AX is a variant form of the nucleosomal protein, histone H2A. H2AX is phosphorylated on its S139 site by ataxia telangiectasia mutated (ATM), a member of the phosphatidylinositol-3 kinase-like kinases (PIKKs), in response to DSBs (Fig.1 A). Phosphorylated H2AX, called $\gamma$H2AX, makes a binding site for many components of the DNA damage response (DDR). The induction of $\gamma$H2AX around DSBs can be detected as ‘$\gamma$H2AX foci’ under a microscope with immunocytochemistry and utilized as a highly sensitive way to monitor the induction and repair of DSBs. Traditional techniques, such as pulsed-field gel electrophoresis or chromosome analysis, usually require Gy-order doses to detect IR-induced DSBs, whereas $\gamma$H2AX analysis can be utilized with a milli Gy-order dose. Therefore, $\gamma$H2AX analysis has become the most popular tool to detect DSBs in radiation biology. However, the formation of $\gamma$H2AX foci does not solely correlate with DSB formation under certain conditions. ATM- and Rad3-related (ATR), another PIKK, is activated in response to replication stress-induced single-stranded DNA (ssDNA), which, in turn, phosphorylates H2AX (Fig.1 B). Therefore, there are some limitations in $\gamma$H2AX analysis to monitor DSBs at least in S-phase cells.

Oxidative stress induces $\gamma$H2AX in a different manner to IR

Non-homologous end-joining (NHEJ) is the prominent pathway for DSB repair in mammalian cells. To better understand the roles of NHEJ in DDR to IR in humans, we characterized a series of mutant cell lines deficient for NHEJ-related genes generated by gene targeting in the human cell line HCT116 [1]. XRCC4 is one of the key components of NHEJ and the XRCC4-deficient (XRCC4$^{-/-}$) cells are seriously incompetent in the repair of DSBs. Consistent with the close relationship between $\gamma$H2AX foci and DSBs, X-ray-induced $\gamma$H2AX foci disappeared more slowly in XRCC4$^{-/-}$ cells than in parental HCT116 cells [1]. Remarkably, $\gamma$H2AX induced by oxidative stress in H$_2$O$_2$-treated HCT116 and XRCC4$^{-/-}$ cells displayed several different features from those induced by IR [2]. Firstly, oxidative stress-induced $\gamma$H2AX commonly appeared as gross nuclear-wide immunoreactive signals but did not as distinct ‘foci’ (Fig.2 A-L). Secondly, oxidative stress-induced $\gamma$H2AX decreased as fast in NHEJ-deficient XRCC4$^{-/-}$ cells as in NHEJ-proficient HCT116 cells (Fig.2 M, N). If $\gamma$H2AX is correlated with DSB formation and repair, it would decrease more slowly in XRCC4$^{-/-}$ cells than in HCT116 cells. Lastly, $\gamma$H2AX appeared in a biphasic mode following oxidative stress in both HCT116 and XRCC4$^{-/-}$.
cells (Fig.2). Reinduction of γH2AX foci was a unique observation for cells exposed to H2O2 and was not observed in cells exposed to IR. This result suggested that γH2AX foci observed in H2O2-treated cells must be induced by a different mechanism than those in cells exposed to IR.

**ATR is the major kinase inducing γH2AX following oxidative stress**

ATM and ATR are the main kinases that phosphorylate H2AX in the damage response to DSBs or ssDNA, respectively (Fig.1 A and B). To examine the participation of these kinases in the damage response following the H2O2 (200 μM) treatment, we analyzed the induction of γH2AX in the presence of specific inhibitors of ATM or ATR (Fig.3). When cells were cultured with a ATM inhibitor (ATMi, KU-55933), γH2AX was underexpressed immediately after the 1 h treatment (0 h), while its expression was equivalent to that in control cells without a kinase inhibitor 2 h after the treatment or later. The reappearance of γH2AX was not affected by ATMi 24 h after the treatment. On the other hand, the phosphorylation of H2AX in responses to H2O2 was largely disturbed by the presence of ATR inhibitor (ATRi, VE-821). A very large fraction of cells at 24 h after the H2O2 treatment with ATRi became detached from glass coverslips during the immunostaining process of the fixed sample, most probably because of cell death. Western blot analyses of prominent substrates for ATM or ATR showed that ATR was activated but ATM was not by 200 μM H2O2 (details are in [2]). These results demonstrated the predominant participation of ATR, rather than ATM, in the oxidative stress response. Cell cycle analysis by pulse labeling of S-phase cells with 5-ethynil-2'-deoxyuridine revealed that the γH2AX was induced in both S- and non-S-phase cells following the H2O2 treatment (details are in [2]). Therefore, γH2AX must be induced in different mechanisms in response to replication stress and to oxidative stress (Fig.1 B and C).

**Conclusion**

The present study demonstrated the biphasic induction of γH2AX following oxidative stress-induced by the H2O2 treatment. The initial and later inductions of γH2AX were primarily mediated by ATR and both were unlikely to associate with DSBs. Reinduction of γH2AX 24 h later can be associated with cellular senescence, because oxidative stress closely associates with organional aging. Oxidative stress also contributes to many aspects in biological responses to IR, such as inflammation. Hence, we cannot rule out the possibility that oxidative stress induced γH2AX affected the results of γH2AX analysis following oxidative stress. To improve the reliability of γH2AX analysis, further understanding for γH2AX induction following oxidative stress is needed.

**References**


Introduction

Aircrews are exposed to elevated levels of cosmic radiation at aviation altitudes because the dose rate of cosmic radiation increases with altitude. The ICRP has recommended in ICRP Publication 60 that the cosmic radiation exposure of commercial jet crews should be included in the category of occupational exposure. The Radiation Council of the Japanese government established a guideline for the management of cosmic radiation exposure of air crews in 2006. From 2007 NIRS started to help airlines to assess the annual dose of their aircrews to follow the guideline. The aviation route doses which are used for the assessment of the aircrew annual effective doses are calculated by the NIRS as a contracted service using an originally developed program called “JISCARD EX” [1]. The guideline requests the airlines to prepare an appropriate correspondence to the additional dose due to solar flares by using a forecasting method such as a space weather forecast. Actually, outbreaks of solar flares do not directly cause an additional dose to aircrews, but a dose increasing phenomenon called Ground Level Event (GLE), which occurs when energetic solar particles emitted by solar flares arrive at the Earth’s atmosphere, cause it. GLE has occurred once a year during the past 70 years on average.

The purposes of this study are to improve the reliability of the evaluation of aircrew doses using numerical model calculations and to prepare for evaluation of the additional dose at a GLE outbreak. From 2007 we started cosmic radiation measurements at the Mt. Fuji automated weather station (Fig.1) with the support of the NPO “Valid Utilization of Mt. Fuji Weather Station”. This weather station is 3,776 m above sea level, and is a suitable observatory for high-altitude cosmic radiation studies [2, 3]. From 2010, construction began for a radiation monitoring system that can be used for annual continuous observations at the Mt. Fuji automated weather station. In this highlight, we outline the radiation monitoring system and summarize some of the monitoring data obtained by it in 2013.

Materials and methods

The radiation monitoring system was installed on the second floor of the Mt. Fuji automated weather station. It consists of a moderator-type neutron rem meter (FHT 762 WENDI-2, Thermo Fisher Scientific Inc.), a data logger / controller (NM10, Melex Ltd.), a long-distance wireless local area network (WLAN) router, a directional Uda-Yagi antenna and two types of battery power units with Li-ion batteries and lead-acid batteries, respectively. A photo of the radiation monitoring system is shown in Fig.2. The Wendi-2 is suited to measure cosmic-ray induced neutrons (cosmic neutrons) since it responds to a wide energy range from 25 meV to 5 GeV. A long-distance WLAN receiving system composed of the long-distance WLAN router, the directional Uda-Yagi antenna, and a data receiving program which runs on a Windows PC and a CATV Internet connection device was installed in the Fuji observatory of Nagoya University. The two directional Uda-Yagi antennas were precisely set oppositely facing each other. The distance between the Mt. Fuji automated weather station and the Fuji obser-
Results and discussion

Fig. 3 shows the neutron count per hour (cph) measured using the WENDI-2 at the Mt. Fuji automated weather station from August 22, 2013 to March 12, 2014, and the atmospheric pressure there during the same period. The atmospheric pressure data were obtained from the Japan Meteorological Agency web site. The reason for measuring neutrons in this study is that cosmic neutrons are the main contributor to cosmic radiation exposure. In the measurement period, the measured neutron count rate changed with approximately 20% variance between 100 - 150 cph. In general, we consider the neutron count rate changes gently. A negative correlation is clearly seen between the neutron count rate and the atmospheric pressure. This is because the absorption in the atmosphere of cosmic neutrons increases as the upward atmosphere thickens.

Monthly averages of the change in a day of the neutron count rate from August 2013 to January 2014 are shown in Fig. 4. A difference in neutron count rate level according to the month was apparent. No correlation was seen between the neutron count rate and the change in a day.

At present, the atmospheric pressure seems to have a dominant effect on the change of the neutron count rate. We intend to investigate other factors which may be buried in the atmospheric pressure influence in the future. The current monitoring system may detect a GLE with more than 40% of increases in counting rate per hour. We think that the accomplishment of the annual continuous monitoring is a prior problem now because no one knows when a GLE will occur.

References

Introduction

Excess relative risk (ERR) is categorized as a relative excess measure and is often used as an effect measure in the analyses of radiation epidemiological data. If ERR values have been derived from a linear dose-response analysis, they are often expressed as a relative increase in rates per unit dose, e.g., ERR per Gy. In this highlight, we refer to ERR per Gy as ERR for simplicity.

Numerous studies have shown that the ERR of solid tumors per unit of radiation is much higher in children than adults, meaning children are more sensitive to the carcinogenic effects of radiation than adults. The exceptions to this finding are cancers of several sites. However, the dose-response relationship between radiation and the long-term effects of radiation in childhood are poorly quantified. In addition, the small numbers of subjects in each study have limited evaluation of the risk of second malignant neoplasms (SMNs) after radiotherapy.

Previously, we evaluated the risk of SMN using a meta-analysis of nine studies in which ERR estimates were presented [1]. However, such a small number of eligible studies available restricted detailed quantitative evaluations. Additional studies in which risk estimates were expressed in terms of relative risks such as rate ratio, hazard ratio, or odds ratio per several dose categories were available; however, due to non-comparability of data, these studies were not included in the meta-analysis.

The objective of the present work was to develop a method to calculate ERR estimates from other forms of risk estimates. Using this new method, we conducted a meta-analysis of 26 studies and examined additional detailed evaluations of SMN risk according to study characteristics. We especially focused on age-related variability of radiation effects.

Method

We proposed a method for estimating ERR from studies in which relative risk estimates such as odds ratio, incidence rate ratio, or hazard ratio are calculated by dose category. We consider a study in which relative risk estimates for several dose categories are available. We assume the relative risk estimate, confidence limits, and representative dose for each dose category are available. The standard error (SE) of the estimate is calculated from the relative risk estimate and confidence limits. Regarding the relative risk estimate and representative dose as data, we fit a linear regression model in which the intercept is fixed at 1 and the slope of the model corresponds to ERR (Fig.1). The SE of the estimate is obtained through a parametric bootstrap method.

Meta-analysis of second malignant neoplasms risk in childhood cancer survivors

Relevant studies were identified by a systematic search of the literature using the PubMed database (from 1950 to 2009). We used Medical Subject Headings (MeSH), a large controlled vocabulary developed for indexing journal articles and books on the scientific literature. This approach allows for the systematic retrieval of studies relevant to the topic of interest. The search was limited to articles published in English to ensure comprehensibility.

Fig. 1 Illustration of our method: The slope of the regression model corresponds to ERR.
life sciences, to retrieve studies indexed using the MeSH terms “neoplasms, second primary” and “radiotherapy.” After performing the initial search using these MeSH terms, additional studies were retrieved with a standard keyword search using the terms “paediatric/pediatric” or “childhood.” The computer search was supplemented by hand-searching reference lists of already retrieved papers. The titles and abstracts of all studies were scanned to exclude irrelevant publications.

The following inclusion criteria were used to determine the studies for the meta-analysis: (1) the endpoint of the study should be SMN risk among childhood cancer survivors, (2) the study design should be either a cohort study or a case-control study, (3) risk estimates should be expressed in terms of ERR (or ERR was calculable from category-specific risk estimates including rate ratio, hazard ratio, and odds ratio), and (4) sufficient data should be present in the publications to enable estimation of the SE of the ERR estimates. If the publication was an earlier report of data that was subsequently updated in another article, it was excluded from the analysis.

Generally, logistic regression analysis is utilized for case-control studies, and Poisson and Cox regression analyses are utilized for cohort studies, and risk estimates obtained from these regression models are stated as an odds ratio, rate ratio, or hazard ratio. Following our previous study that included randomized controlled trials (risk ratio), case-control studies (odds ratio), and cohort studies (rate ratio), we treated ERR estimates from odds ratios, rate ratios, and hazard ratios equally and included them in the meta-analysis.

Result

We selected 198 studies in the first step of the systematic literature search using MeSH terms and keywords. Of these, we excluded 180 studies that did not satisfy all of the inclusion criteria. We added 10 studies to the pool of eligible studies by conducting a hand search. Two studies, one that used data reported earlier but included in a subsequent article and one that used identical data, were excluded. Eventually, we identified 26 epidemiological studies. Among these studies, ERR estimates were available for 15 studies, and ERR estimates were calculable for 11. There is a study in which ERR estimates for more than one site are available, and we ultimately included 27 ERR estimates in the meta-analysis gleaned from the 26 eligible studies.

The results from the meta-analysis are presented in Table 1. The overall ERR using all studies included in the meta-analysis was 0.60 (95% CI: 0.30, 1.20; n = 27). The overall estimate of ERR from the cohort studies was 1.22 (95% CI: 0.45, 3.33; n = 13), and that from the case-control studies was 0.30 (95% CI: 0.12, 0.73; n = 14). The overall estimate of ERR from European studies was 0.74 (95% CI: 0.30, 1.83; n = 20), from the North American studies was 0.28 (95% CI: 0.07, 1.17; n = 5), and from both European and North American studies was 0.46 (95% CI: 0.06, 3.10; n = 2). The overall estimates of ERR according to SMN sites were as follows: thyroid, 3.01 (95% CI: 1.08, 8.35; n = 7); bone and soft tissue, 0.48 (95% CI: 0.03, 7.30; n = 4); breast, 0.31 (95% CI: 0.16, 0.59; n = 4); brain, 1.51 (95% CI: 0.10, 23.09; n = 4); and leukemia, 0.38 (95% CI: 0.004, 37.88; n = 2). The overall estimate of ERRs available in the original papers was 0.99 (95% CI: 0.43, 2.25; n = 16), and that calculated by the proposed method was 0.25 (95%CI: 0.08, 0.84; n = 11).

In the meta-regression model, which included age at primary cancer diagnosis as a covariate of main interest, the regression coefficient was -0.159 (95% CI: -0.293, -0.024), indicating a significant decrease in ERR with an increase in the age at primary cancer diagnosis (0.85 times [95%CI: 0.75, 0.98] per year). The analysis, which included the site of the SMN as a covariate in addition to the age at primary cancer diagnosis, also revealed a trend of decreasing ERR with increasing age at primary cancer diagnosis (0.92 times [95%CI: 0.78, 1.08] per year); however, this trend was not statistically significant.

Discussion

We proposed a method for calculating ERR estimates from dose category-specific risk estimates. Using this method, the number of studies included in the meta-analysis was increased from 15 to 27, thus enabling us to perform a separate analysis based on type of SMN. We could also quantify the dependence of ERR on the age at primary cancer diagnosis.

The increased number of studies included in the meta-analysis enabled us to evaluate the separate estimate for each type of SMN. The risk estimates of childhood thyroid cancer exposed to radiodine after the Chernobyl accident have varied from 5.5 (95% CI: 3.1, 9.5) to 8.4 (95% CI: 4.1, 17.3), depending on the risk model[2]. The pooled risk estimate from five cohort studies of childhood thyroid cancer including studies of atomic bomb survivors and medically irradiated children is 7.7 (95% CI: 2.1, 28.7) [3]. In our work, the overall estimate for thyroid was 3.01 (95% CI: 1.09, 8.35; n = 7), and compatible with these studies.

With the methods developed to calculate ERR, we have conducted a meta-analysis that includes a greater number of studies about SMN risk among childhood cancer survivors than was previously possible. From the detailed evaluation, some factors which may explain heterogeneity were suggested, such as age at which the cancer is first diagnosed. For further evaluation of how the age impacts ERR, further studies should include patients who are irradiated in adulthood in addition to childhood.

Table 1 Results from the meta-analysis

<table>
<thead>
<tr>
<th>ERR estimate</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Overall (n = 27)</td>
<td>0.60</td>
</tr>
<tr>
<td>Cohort studies (n = 13)</td>
<td>1.22</td>
</tr>
<tr>
<td>Case-control studies (n = 14)</td>
<td>0.30</td>
</tr>
<tr>
<td>European studies (n = 20)</td>
<td>0.74</td>
</tr>
<tr>
<td>North American studies (n = 5)</td>
<td>0.28</td>
</tr>
<tr>
<td>European and North American (n = 2)</td>
<td>0.46</td>
</tr>
<tr>
<td>Thyroid (n = 7)</td>
<td>3.01</td>
</tr>
<tr>
<td>Bone and soft tissue (n = 4)</td>
<td>0.48</td>
</tr>
<tr>
<td>Breast (n = 4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Brain (n = 4)</td>
<td>1.51</td>
</tr>
<tr>
<td>Leukemia (n = 2)</td>
<td>0.38</td>
</tr>
<tr>
<td>ERR available (n = 16)</td>
<td>0.99</td>
</tr>
<tr>
<td>ERR calculated (n = 11)</td>
<td>0.25</td>
</tr>
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References