Research for Radiation Protection

Yoshiya Shimada, Ph.D.
Deputy Director of Research Center for Radiation Protection
E-mail: shimada.yoshiya@qst.go.jp

1. 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: the Center has been appointed as a Collaborating Center by the International Atomic Energy Agency and the appointment lasts until 2018.

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 the R&D Team for Biospheric Assessment for Waste Disposal.

2. FY2015 activities
2.1. Radiobiology for Children's Health Program
1) Background and objectives of research
In this era of low birthrate and prolonged longevity in Japan, concerns about the safety of fetuses and children with respect to radiation protection 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) Main results
- The relative biological effectiveness of neutrons, from exposure during childhood, was determined experimentally for cancer risk of kidney and brain.
- Experiments was continued to investigate the effect of repetitive radiation exposure, giving reduction factors related to fractionation of radiation exposure for gamma rays.
- Novel findings were made on characteristic mechanisms of carcinogenesis associated with childhood exposure to radiation, including genetic mutations and early responses.
- Genomic analysis was conducted, for the first time in the world, on uranium-induced cancer in an animal model and a genetic alteration was found.
- Three articles, to which members of the Program had contributed as authors, were cited in ICRP Publication 131 Stem Cell Biology with Respect to Carcinogenesis Aspects of Radiological Protection (2015).

2.2. Radiation Risk Reduction Research Program
1) Background and objectives of research
Susceptibility to radiation-induced malignancies differs depending on the individuals. Variable efficiencies of the DNA repair function resulting from single nucleotide polymorphisms (SNPs) located in genes for DNA repair-related proteins are thought to be one of the factors that cause individual differences in radiation sensitivity. In addition, there is evidence suggesting that individual radiation sensitivity can be modulated by lifestyle practices. These practices include smoking habits which have been shown to elevate an individual’s sensitivity to α-particles. The purpose of this program is to identify factors, whether genetic or 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) Main results
- More radiation-induced micronuclei were observed in mice administered Japanese rice wine (sake) than control mice, with varying degrees in induction of antioxidant activity in the liver depending on the grade of sake.
- Results were summarized on DNA damage repair factors Ku80 and Rad52 and candidates of protein markers of radiosensitivity were listed.
- A combination of mild dietary restriction and radiation-induced adaptive response was effective in reducing genotoxicity of radiation.
- A research perspective was reported to OECD/NEA-CRPPH regarding modification of radiation sensitivity by lifestyle factors including dietary habits.

2.3. Regulatory Science Research Program
1) Background and objectives of research
   Objectives of this program are to investigate the necessary information for development of radiation safety standards and guidelines and to propose scientifically based measures for radiation regulation and policy aiming at a more reasonable system of radiation protection. For such purposes, the scientific knowledge is processed in a suitable form to apply each practice and to provide it to government regulatory agencies and to society.

2) Results
   - Twelve research articles were published regarding protection of radiation from natural sources.
   - Information on research needs for the next mid/long-term plan was actively collected from international organizations including ICRP and IAEA.
   - To establish grounds for deployment in research and other activities, agreements were concluded on research collaboration with overseas organizations and partnerships were strengthened with governmental administration and educational organizations.
   - Experts were dispatched to national councils and other meetings to support setting of standards for radiation safety. For example, in a cross-ministerial meeting on safety of participants in offsite disaster prevention, a proposal was made on training of disaster prevention workers during normal times, which was adopted in the final report.

2.4. R&D Team for Biospheric Assessment for Waste Disposal
1) Background and objectives of research
   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 (TF and K) considering climate change; (2) estimating the effects on microbial activities for the transfer parameters of carbon-14 in soil-plant systems; and (3) collecting the environmental transfer parameters of important radionuclides (Pu, Am, Th and Cl) by ultra-high sensitivity analysis.

2) Main results
   - Regarding development of analysis methods for trace radionuclides, the recommended temperature for soil sample ashing in the nitric acid leaching method was determined for plutonium, which is applicable to other artificial radionuclides (e.g. strontium-90 and americium-241) in soil samples; for seaweed samples, there is no limitation for the ashing temperature.
   - Concentrations of cesium-137 and potassium-40 were measured in leaves of several perennial herbaceous and woody plants and it was clarified that these two radionuclides do not behave similarly in the leaf blade and petiole.

3. Summary of main results of the mid-term plan
3.1. Experimental research for radiation protection of children
   - Information for age-weighting factors of radiation risk was collected through experiments on age dependence of the relative biological effectiveness of neutrons on induction of lung cancer, myeloid leukemia, renal cancer and brain tumor.
   - Reduction factors related to fractionation of radiation exposure were experimentally determined regarding life shortening of young animals exposed to gamma rays or carbon ions.
   - Persistence of uranium exposed during infancy was clarified in a rat model; a novel model was developed in which exposure to uranium induces renal carcinogenesis with an identified genetic mutation.
   - As biological grounds for age-weighting factors for radiation risk, distinct mechanisms of carcinogenesis were revealed between infant and adult animals.
   - These and other results were presented in international meetings including the WHO Collaboration Center Symposium and the International Congress of Radiation Research and cited in important publications issued by UNSCEAR, ICRP, NCRP and WHO (a cumulative total of 11 citations).

3.2. Mechanistic studies aiming at risk reduction
   - High calorie diets, drinking alcohol and hormones were shown to be factors that may modify radiosensitivity of mice.
   - Two amino acids of Ku80 and 8 amino acids at the C terminus of Rad52 were shown to be candidates of biomarkers for individuals with high susceptibility to radiation-induced carcinogenesis.
   - Dietary conditions were identified to be factors that modify efficiency of radiation-induced adaptive response.
   - Artemis, a protein for non-homologous end joining, was shown to have a function that may increase mutation frequency in DNA damage response after low-dose radiation exposure.

3.3. Regulatory science for bridging the gap between scientific findings and society
   - As the main sources of radiation exposure in Japan have been medical radiation and natural radiation even after the Fukushima Daiichi nuclear power plant accident, the current status of, and countermeasures to, natural radiation exposure in Japan were clarified and information on management measures and requirements thereof were conveyed to the regulation authorities.
   - Regarding the important issue of risk of radiation at low dose and low dose rate, mathematical models and pooled analysis were used to more precisely estimate radiation risks, by incorporating the latest epidemiological information and parameter values for the Japanese population.
   - To support risk communication activities conducted nationwide after the Fukushima Daiichi nuclear power plant accident, information sources and methodologies were developed and published.
   - Regarding the environmental effect of the Fukushima Daiichi nuclear power plant accident, exposure levels of wild life and the dose rate of no observed effect were estimated, present-
Highlight

Distribution differences of K and $^{137}$Cs in leaf blade and petiole of herbaceous and woody plant leaves

Keiko Tagami, Shigeo Uchida
E-mail: tagami.keiko@qst.go.jp

Introduction

Large areas in Eastern Japan were contaminated with radiocesium ($^{134}$Cs and $^{137}$Cs) after the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident. Because of the similar chemical reactivities of Cs and potassium (K), $^{40}$K/$^{137}$Cs ratios in aboveground plant tissues are similar. Therefore, it was widely thought that to predict the behavior of radiocesium in plants, K could be used as an analogue of radiocesium for any plant species after uptake through the roots. However, Ban-nai et al. [1] reported that radiocesium concentrations in petiole of three leafy vegetables (lettuce, spinach and komatsuna) were smaller than that in the leaf blade. On the other hand, for sycamore tree, the K concentration in the petiole was found to be higher than that in the leaf blade [2]. These results suggested that petiole and leaf blade would have different K/Cs ratios, which means the elements have different roles in leaves.

In this study, we collected leaves of several perennial herbaceous and woody plants and measured $^{137}$Cs and $^{40}$K concentrations of their leaf blade (LB) and petiole (P) tissues to clarify whether the two elemental distributions were the same or not in other plant species.

Materials and method

The following plants grown wild on the NIRS campus in Chiba City located ca. 220 km south from the FDNPP were collected at various times from 2012 until 2015: giant butterbur (Petasites japonicus, LB and P); Japanese knotweed (Fallopia japonica, LB and P); Japanese dock (Rumex japonicus, LB and P+S (petiole+stem)); ginkgo (Ginkgo biloba, deciduous tree, LB and P); Someiyoshino cherry (Cerasus × yedoensis (Matsum.) A.Vassil. 'Somei-yoshino' deciduous tree, LB and P); and mochi tree (Ilex integra, evergreen tree, LB and P). We have in particular collected giant butterbur regularly since 2012 in the spring. For comparison, we also collected two wild herbaceous plants in Fukushima Prefecture, that is, giant butterbur (LB+P) and momijigasa (Parasenecio delphinitiifolius, LB and P+S) in 2013 and 2015, respectively.

Immediately after the collection, plant specimens were transferred to a laboratory and then, each tissue part was separated as shown in Fig. 1. They were separately washed with tap water to remove dust from the surface; this was done in a washing bowl by changing the water 5 times, and then, finally, the samples were rinsed with reverse osmosis water. All samples were oven-dried to a constant weight at 80°C in an electric oven for at least 2 d to decrease the sample volume. Each oven-dried sample was pulverized and mixed well, and then transferred to a 100-mL plastic container.

Radioactivity concentration in each sample was measured by a Ge detecting system (Seiko EG&G) using 50,000–150,000 s counting intervals. A mixed gamma standard solution (Amersham, QCY-46) was used for an efficiency correction. The $^{137}$Cs activity was decay corrected to the sampling date. Potassium analysis was done based on $^{40}$K because the radionuclide is a nearly perfect indicator of stable K.

Concentration ratios for $^{137}$Cs (CR_Cs) and $^{40}$K (CR_K) were defined as

$$CR_{\text{Cs}} = \frac{C_{\text{LB-Cs}}}{C_{\text{PS-Cs}}} \quad \ldots \quad (1)$$

$$CR_{\text{K}} = \frac{C_{\text{LB-K}}}{C_{\text{PS-K}}} \quad \ldots \quad (2)$$

where $C_{\text{LB-Cs}}$ is $^{137}$Cs concentration in LB, $C_{\text{PS-Cs}}$ is $^{137}$Cs concentration in P (+S), $C_{\text{LB-K}}$ is $^{40}$K concentration in LB and $C_{\text{PS-K}}$ is $^{40}$K concentration in P (+S). Then the discrimination ratio (D) of $^{40}$K/$^{137}$Cs of LB to P (+S) was calculated as $CR_{\text{K}} / CR_{\text{Cs}}$, that is,

$$D = \left( \frac{C_{\text{LB-K}}}{C_{\text{PS-K}}} \right) / \left( \frac{C_{\text{LB-Cs}}}{C_{\text{PS-Cs}}} \right) \quad \ldots \quad (3)$$

Fig. 1 Leaf blade and petiole samples for giant butterbur and ginkgo.
Results and discussion

We judged the source of $^{137}$Cs in plants collected at NIRS to be mostly from the FDNPP accident because global fallout $^{137}$Cs in plants was low in Japan before 2011. Since March 2011, we have been able to measure $^{134}$Cs in many plant samples. In this highlight, however, we only report $^{137}$Cs and $^{40}$K results.

The measured concentrations of $^{137}$Cs and $^{40}$K in LB and P samples for giant butterbur are shown in Fig.2 on a dry weight basis. The $^{137}$Cs decreased for both LB and P in 2012–2014, because bioavailability of radiocesium added to the soil should decrease with time after the deposition due to the aging effect. We previously reported the effective half-lives of $^{137}$Cs in giant butterbur tissues from 2011–2014 and found the average value of 446 d [3]. However, when we compared the data from 2014–2015, no statistical differences were observed, that is, $^{137}$Cs concentrations did not decrease from 2014 to 2015. Thus we assumed that the bioavailable $^{137}$Cs amount in the soil did not change much between 2014–2015.

From the results of Fig.2, we saw $^{137}$Cs concentrations in LB were higher than those in P; however $^{40}$K showed a different tendency. Specifically, CR-Cs and CR-K were calculated using eqs. (1) and (2), respectively, and the results are shown in Fig.3. The CR-Cs values were from 1.2 – 2.1, while the CR-K values were from 0.49 – 0.70. Then we calculated the discrimination ratio D using eq. (3). If D values are equal to one then the leaf blade and petiole or stem do not discriminate Cs from K, and if the value is lower than one then the leaf blade concentration of K is decreased and/or Cs is enhanced in. The D values were from 0.23 – 0.47 with an average value of 0.35. Thus apparently, K and Cs discrimination was observed in the leaf components, LB and P. One of the reasons for the different $^{137}$Cs and K distributions in these leaf parts might be soil re-suspension onto the plant; however, our previous study of plant tissues from the same sampling sites showed that $^{137}$Cs concentration did not increase by re-suspension of soil onto the giant butterbur LB and P [4]. Therefore, the soil re-suspension effect should be negligible.

To allow us to compare the giant butterbur results with other plant species, further measurements were carried out and the results are shown in Table 1. The only exception was found for mochi tree, an evergreen tree species. Because evergreen tree leaves generally have a longer life than deciduous tree leaves or herbaceous plants have, they might have different physiological chemistry and, consequently, the concentration difference may not be clear between the leaf blade and petiole. Therefore, data for mochi tree leaves are not considered further here.

The CR-Cs values for plant species were higher than 1 (1.2 – 4.1). The tendency was the same not only for the plant samples collected at NIRS but also for samples collected in Fukushima Prefecture. We also used reported data [1] and calculated the CR-Cs for comparison; the value ranged from 1.4 – 2.1 showing the same tendency as the present study.

For $^{40}$K, on the other hand, CR-K values were lower than 1, not only for giant butterbur but also for other plant species, and K concentrations in P were higher than those in LB. The D values were calculated to be lower than 1. These results suggested that Cs and K did not behave similarly in these specific areas of leaf tissues, leaf blade and petiole. We previously reported Cs and K distribution differences between stems and leaves of perennial herbaceous plants; thus Cs and K roles in a plant might be different. From these results we concluded that to understand the detailed radiocesium fate in plants, K measurement results should not be used as an analogue.

References

Table 1. CR-Cs, CR-K and D values of seven plant species.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Collection date</th>
<th>CR-Cs LB/P(+S)</th>
<th>CR-K LB/P(+S)</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese Knotweed</td>
<td>3-Sep-14</td>
<td>-</td>
<td>0.40</td>
<td>-</td>
</tr>
<tr>
<td>Japanese dock</td>
<td>1-May-15</td>
<td>1.3</td>
<td>0.54</td>
<td>0.41</td>
</tr>
<tr>
<td>Giant butterbur</td>
<td>27-Apr-15</td>
<td>1.3</td>
<td>0.84</td>
<td>0.62</td>
</tr>
<tr>
<td>Momijigasa</td>
<td>24-May-13</td>
<td>1.2</td>
<td>0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>Mochi tree</td>
<td>17-Jun-14</td>
<td>2.1</td>
<td>0.95</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*$^a$Samples were collected in Fukushima Prefecture.
The influence of ashing temperature on the determination of Pu in soil and biological samples

Zhongtang Wang, Jian Zheng, Keiko Tagami, Shigeo Uchida
E-mail: wang.zhongtang@qst.go.jp

Introduction
Plutonium is the second element in the transuranium element series. The current existence of Pu in the natural environment is due to human nuclear activities, such as nuclear weapon test explosions, nuclear industry operations and accidental releases. Global fallout, resulting from extensive atmospheric nuclear weapon tests in the last century, is the dominant Pu source in the environment. In recent years, there has been considerable concern regarding the behavior of global fallout Pu in the environment because of the radiotoxicity associated with its alpha-emitting radioisotopes ($^{238}$Pu, $^{240}$Pu).

Attention has also been given to using Pu as a tracer to study geochemical processes, for example, soil erosion, sediment dating and desertification studies. In all these applications, an inevitable analytical operation is to transfer Pu from environmental samples (e.g. soil, sediment and biological samples) into a liquid which is compatible with the subsequent chemical treatment. In the literature, many methods have been presented to achieve this, such as, the nitric acid leaching method, total digestion method and alkali fusion method. Among these methods, nitric acid leaching is the most popular one because it is simple, fast and effective. Normally, the nitric acid leaching analytical method consists of four steps: ashing, acid leaching, Pu separation and Pu measurement. The ashing step is intended to destroy any organic matter in the samples that would have a negative impact on the Pu separation step. Different ashing temperatures, from 400 to 900°C, have been used by different researchers. Various ashing temperatures may cause additional uncertainty in Pu analysis. For example, low temperatures may not decompose the organic matter thoroughly, while high temperatures may produce some refractory particles. Therefore, an appropriate ashing temperature should be identified and accepted by researchers to improve the reliability and accuracy of the nitric acid leaching method.

Thus, in this study, efforts were made to investigate the effect of ashing temperature on accurate determination of Pu using the nitric acid leaching method, for soil and biological samples. Furthermore, an optimum temperature was recommended for sample ashing.

Experimental
One seaweed (IAEA-446) and two soil (IAEA-soil-6 and IAEA-375) standard reference materials were used to in the experiment. 1.5 – 2 g soil samples and approximately 5 g seaweed samples were weighed and ashed in a muffle furnace (FUW 253PA, Tokyo). The ashing temperature was set from 375 to 600°C, and each temperature was used to ash three replicate samples for analysis. After ashing, a well-established two stage anion chromatographic chemical separation method was utilized for sample preparation [1]. Specifically, 20 mL conc. HNO$_3$ was used to leach Pu from a soil sample by heating the sample-HNO$_3$ mixture in a closed PTFE vessel at 160°C for 4 h. After filtration and adjusting Pu to the tetravalent state, the sample was loaded onto a preconditioned 2 mL AG 1 × 8 resin column, which was subsequently washed with 50 mL 8M HNO$_3$ and 30 mL 10 M HCl to remove U, Fe, Pb and Th. Pu was eluted from AG 1 × 8 with 40 mL 0.1 M NH$_4$I-8.5 M HCl solution, followed by sample evaporation, organic matter decomposition (adding 1 mL aqua regia and heating to dryness, repeated twice) and dissolution in 4 mL conc. HCl(H$_2$O$_2$) solution. The sample was then transferred to a preconditioned 2 mL AG MP-1M resin column, where matrix elements like U and Th were further removed by washing with 20 mL 8M HNO$_3$ and 8 mL 10 M HCl. Afterwards Pu was eluted from AG MP-1M resin with 16 mL conc. HBr. After the sample was heated to dryness, 1 mL ultrapure HNO$_3$ was added and heated to dryness again to remove any trace of HBr. Finally, the residue was dissolved in 0.8 mL 4% HNO$_3$ for ICP-MS measurement [2].

Results and discussion
For soil samples, the Pu analytical results of IAEA-soil-6 and IAEA-375 are plotted in Fig.1 and Fig.2, respectively [3]. In Fig.1, for samples ashed at temperatures not exceeding 450°C, the $^{239-240}$Pu activities were generally consistent with the reported range: 0.96 – 1.11 mBq/g. As the ashing temperature increased, however, an obvious decreasing trend of $^{239-240}$Pu was observed. The lowest activity was found in samples ashed at 600°C, in which only 62% of the Pu was recovered, compared to the certified $^{239-240}$Pu activity (1.035 mBq/g). In contrast to the $^{239-240}$Pu activities, the $^{240}$Pu/$^{239}$Pu atom ratios plotted in Fig.1 did not show any significant difference at various ashing temperatures, and all the ratios were within the reported range. This may indicate that no isotopic discrimination occurred during
As discussed above, both soil standard reference materials had a decreasing trend for 239+240Pu activity when the ashing temperature exceeded 450°C, indicating that a smaller Pu content was measured in these samples. Since all the experimental conditions were the same except ashing temperature, it was hypothesized that the Pu loss in these soil samples may be attributed to high temperature ashing by forming some refractory fractions in which some portion of the Pu was trapped and could not be leached out by HNO3. To verify this hypothesis, X ray diffraction (XRD) analysis was performed to examine the chemical composition change in soil samples after ashing. The XRD results of non-ashed, 400°C ashed and 600°C ashed soil samples showed that new crystalline phases were generated in the 600°C ashed samples. These new phases were further identified as plagioclase-like silicate materials which are known to be insoluble in HNO3.

To validate the hypothesis, various combinations of leaching/digesting conditions, including conc. HNO3, HNO3-HF, and HNO3-HF-HClO4, were utilized to treat IAEA-soil-6 samples after ashing at 550°C. The 239+240 Pu activity obtained from the conc. HNO3 leaching method was 0.67 ± 0.02 mBq/g, significantly lower than the reported range: 0.96 – 1.11 mBq/g. For the HNO3-HF leaching and HNO3-HF-HClO4 digestion methods, silicate fractions formed during ashing were dissolved by HF, resulting in the 239+240 Pu activity being within the reported range: 0.96 ± 0.03 mBq/g for HNO3-HF leaching; and 0.99 ± 0.02 mBq/g for HNO3-HF-HClO4 digestion. Consequently, based on the above discussion, it was confirmed that high ashing temperatures (500 – 600°C), can lead to formation of some refractory silicates which remain insoluble in conc. HNO3, and that results in Pu loss in the HNO3 leaching method.

For seaweed samples, only two temperatures were assessed (450 and 600°C), with the results shown in Table 1. For both temperatures, the Pu activities and atom ratios were all consistent with the certified values, indicating that no ashing temperature effect was observed. The contradictory behavior to that of soil samples might be attributed to the different silicate concentrations in soil and seaweed materials. Soil is well to be rich in silicon, with the proportion up to one third, while the silicate fraction in the investigated seaweed sample is only 0.042%. Therefore, for seaweed samples, high temperature ashing (> 450°C) does not lead to Pu loss for the conc. HNO3 leaching method.

Overall, the results of soil samples showed less Pu extractability with HNO3 when the ashing temperature exceeded 450°C, due to the formation of plagioclase-like refractory fractions. The findings of this study suggest that the temperature for soil sample ashing in the HNO3 leaching method should be controlled below 500°C, and 450°C is recommended. This suggestion is also useful for the determination of other artificial radionuclides (e.g. 90Sr, 241Am) in soil samples. But for seaweed samples, there is no limitation for the ashing temperature.

Table 1  Pu analytical results of the ashing temperature experiment for IAEA-446 seaweed samples (n=3)

<table>
<thead>
<tr>
<th>Ashing temperature</th>
<th>450°C</th>
<th>600°C</th>
<th>Certified value</th>
</tr>
</thead>
<tbody>
<tr>
<td>239+240Pu activity (mBq/g)</td>
<td>0.026 ± 0.001</td>
<td>0.025 ± 0.003</td>
<td>0.024 ± 0.002</td>
</tr>
<tr>
<td>240Pu/239Pu atom ratio</td>
<td>0.222 ± 0.003</td>
<td>0.230 ± 0.007</td>
<td>0.220 ± 0.006</td>
</tr>
</tbody>
</table>

References
Using genetic analysis to examine T cell lymphomas arising in mice irradiated with carbon ions or gamma rays

Benjamin J. Blyth

Introduction

One of the key advantages of carbon ion radiotherapy is the reduction of radiation deposition in normal tissue ahead of the target volume. The reduction in second cancer risk associated with this dose reduction is, however, dependent on the relative cancer risk associated with this radiation exposure compared to traditional radiotherapy modalities. In this project [1], we examined T cell lymphomas which arose in mice exposed starting from 1 week old to a mono-energetic carbon ion beam (290 MeV/u) produced at the Heavy Ion Medical Accelerator at Chiba (HIMAC) at the NIRS, such that the whole-body received the low LET (13 keV/μm) deposition ahead of the Bragg Peak recapitulating the exposure of normal tissue ahead of an irradiated tumour. We examined both single exposures of 4 – 4.8 Gy as well as the same total dose delivered over four once-weekly fractions. Our aim was to determine whether the radiation-induced tumours from these exposures showed different cancer-initiating events from tumours arising following a standard gamma ray exposure at the same dose.

Induction of Lymphomas by Gamma or Carbon Irradiation

When comparing a single exposure of 4 Gy, carbon ions induced a significantly increased frequency of T cell lymphomas (cancer of the thymus originating from immature T cells), with a significantly reduced T cell lymphoma-free lifespan, an indication of a higher RBE for carbon ions, even at the low LET relevant for normal tissue exposure (Fig.1). The effect of

---

**Fig.1**  T cell lymphoma-free survival of mice irradiated with gamma rays or carbon ions. The Kaplan-Meier estimator is plotted for the five irradiation groups with censored cases (causes of death other than TL) marked with crosses. Curves sharing the same letter designation (a, b or c) are not significantly different by pairwise log-rank tests (P > 0.05).
fractionation for carbon ion irradiation was complex, with the 4 Gy and 4.8 Gy doses at the inflection point for efficient induction of early lymphomas instead of late-occurring solid tumours. However, when the 4 Gy carbon dose was fractionated mimicking a clinical regimen, the risk was the same as for a single dose of gamma rays, suggesting that normal tissue can be partially protected from second cancers using this method that is routinely employed to prevent acute tissue reactions.

Genetic Analysis of Tumour Suppressor Genes

We selected T cell lymphomas from a cohort of 100 carbon ion irradiated mice, based on records in J-SHARE (Japan-Storehouse of Animal Radiation Experiments) an experimental and pathology archive of lifetime radiobiology experiments at NIRS. For comparison, we also examined 16 tumours from gamma irradiated mice.

A selection of the carbon tumours (n=20) and all of the gamma tumours (n=16) were examined by whole genome DNA copy number analysis to identify regions of the genome which had suffered deletions or amplification events. Identifying which regions of the genome had been lost allowed us to characterise candidate tumour suppressor genes within the deleted regions. In both carbon and gamma tumours, deletions were identified over regions containing known T cell tumour suppressor genes, including Pten, Bcl11b, Ikrz1, Trp53, Cdkn2a/Cdkn2b and Notch1.

Allelic loss on the chromosomes harbouring Pten, Bcl11b, Ikrz1 and Trp53 genes was then examined across the whole cohort of tumours. Those genes which are known to frequently suffer DNA mutations were also screened by sequencing of mRNA transcripts via reverse-transcription PCR. Many of these gene alterations were mutually exclusive, as they converge on important T cell developmental networks, removing any advantage from multiple hits to the same pathway. Although some significant differences were observed in the frequency of the competing inactivation modes (loss of a Bcl11b allele was more common in carbon tumours, while Pten loss was more common in gamma tumours), on the whole, both radiation types showed cancer-initiating mutations in canonical T cell lymphoma genes.

Interstitial Chromosomal Deletions

Many of the DNA copy number aberrations were small (less than a few million basepairs) and often occurred within tumour suppressor genes, resulting in loss of gene expression. These are known to arise in many cases by illegitimate recombination events, whereby cryptic signal sequences are recognised by the cell’s normal gene rearrangement machinery and undergo unintended recombination events. Larger events included whole chromosome loss, or loss of a chromosome at a breakpoint to the end of the chromosome. However, it was observed that large interstitial deletions, or a loss of more than 5 million basepairs of DNA from within the chromosome, was more common in the carbon tumours than those from mice irradiated with gamma rays (Fig 2). These deletion events are more complex, requiring the DNA to be broken in two distant locations and for the intervening DNA to be lost before repair of the distant ends. Genes in the middle of a chromosome are normally more resistant to deletion, yet it appears that even at the lower LET in the plateau region, heavy ion irradiation is more effective at inducing these lesions.

Interestingly, most of these large interstitial deletions did not involve any of the T cell lymphoma tumour suppressor genes and are likely hallmarks of the heavy ion radiation exposure, but are not the causal events. This is consistent with radiation acting as a genotoxin to introduce cancer-initiation mutations, as well as a promoter of cells harbouring DNA damage to expand in tissues which need to recover from large-scale radiation-induced cell death.

Conclusions

The efficacy of strategies to use heavy ion irradiation to spare normal tissues from acute radiotoxicity may also reduce the risk of future second cancers in surrounding normal tissues. However, the benefit depends not only on the dose reduction, but also on the biology of how the heavy ions interaction with healthy tissues to increase cancer risk. We showed here that even at low LET, carbon ions were more effective at causing radiation-induced tumours in a mouse model, and that they may harbour unique signatures of the exposure. However, we also saw that for this tumour type, the initiating events were similar to those after photon irradiation, and that large interstitial deletions might be an additional risk for second cancer induction, but was not the primary cause of tumour suppressor gene loss. Future studies in other tumour types and for alternative exposure parameters will help establish these lesions as bone fide markers of heavy ion exposure.

Reference

The influence of age at exposure on genetic alterations in radiation-induced mouse T-cell lymphomas

Masaaki Sunaoshi

1. Introduction

It has been considered that children are more sensitive to radiation-induced cancer than adults, because i) children’s tissues and organs are growing and developing rapidly, and ii) they have more time for radiation-induced tumors to manifest. Epidemiology studies of the atomic bomb survivors from Hiroshima and Nagasaki show that the risk of radiation carcinogenesis is higher in exposed children than adults, with leukemia showing the greatest modification by age. Our animal experiments assessing radiation risk depending on age at exposure show similar results to the human studies. However, the mechanism of the age dependency is still unclear, making it necessary to reveal the molecular mechanisms which increase the risk of radiation carcinogenesis at a young age. However, due to the small number of tumors associated with childhood radiation exposure available for study, the inability to distinguish radiation-induced tumors from spontaneous tumors, and the retrospective nature of such human studies, very little is actually known about molecular characteristics associated with age at exposure. In this study, we examined radiation-induced T-cell lymphomas, a mouse model of human acute lymphoblastic leukemia, as a way to investigate any molecular mechanisms that might be dependent on the age at exposure.

In this highlight, we show that age at irradiation might influence the targets and mechanisms of tumor suppressor gene inactivation in radiation-induced T-cell lymphomagenesis, or favor particular tumor pathways due to age-specific selection pressures [1].

2. Results

2.1. T-cell lymphoma incidence

To characterize age-specific molecular changes in tumors, we analyzed T-cell lymphomas induced by 1.2 Gy whole-body X-ray irradiation of female B6C3F1 mice for 4 consecutive weeks starting at 1, 4 or 8 weeks of age (Fig.1). As the thymus continues to grow between birth and puberty, and then begins to atrophy, the three age groups chosen represent the respective growth stages of the thymus and correspond to the infant (1–4 weeks old), adolescent (4–7 weeks old), and young-adult (8–11 weeks old) periods in B6C3F1 mice. The incidences of T-cell lymphoma in infant-, adolescent-, and young adult–irradiation groups were 26% (16/62), 34% (17/50) and 22% (11/50), respectively. The incidence was the lowest in the oldest age group, although the differences among the three age groups (all of which were still young when compared with the average B6C3F1 lifespan of about 124 weeks) were not statistically significant. Furthermore, comparison of other metrics such as lifespan of mice with lymphoma, tumor latency, and tumor weight did not show statistically significant differences among the three groups.

Irradiation group X-ray 1.2 Gy × 4 (Weekly)
Infant \( n = 62 \)
Adolescent \( n = 50 \)
Young adult \( n = 50 \)

Fig.1 Experimental design of T-cell lymphoma induction.

Mice were observed daily until moribund, when they were sacrificed by exsanguination under terminal isofluorane anesthesia, and they were assessed for the incidence of T-cell lymphoma.

2.2. Age at exposure–dependent distribution of LOH and copy-number alteration in lymphomas

Since loss of heterozygosity (LOH) is frequently accompanied by inactivation of tumor suppressor genes in radiation-induced tumors, we determined the frequency of LOH at microsatellite markers flanking the \( Cdkn2a \) (chromosome 4), \( Ikaros \) (chromosome 11), \( Bcl11b \) (chromosome 12) and \( Pten \) (chromosome 19) candidate tumor suppressor genes implicated in radiation-induced T-cell lymphoma in our previous studies [2]. LOH can occur as a result of chromosomal deletion (with loss of DNA copy number) or recombination/mis-segregation (without copy number change). Thus, to reveal the LOH mechanism, we used array-based comparative genome hybridization (CGH) to distinguish between copy number loss- and copy number neutral-LOH on these chromosomes. In particular, we noted that the nearest markers of \( Cdkn2a \), \( Ikaros \) and \( Pten \) loci showed signs of the
LOH frequency changing with age at exposure, the LOH analysis was extended to markers along the length of chromosomes 4, 11 and 19. Conversely, the Bcl11b locus did not show any age-dependent changes in LOH frequency, and thus was not studied further.

LOH on chromosome 4 always included the marker nearest Cdkn2a and was either by interstitial deletion (of one or both copies) or copy number–neutral LOH along the whole chromosome. The frequency of LOH or deletions including the Cdkn2a locus was higher in the young adult–irradiation group than in both of the younger groups. LOH on chromosome 11 always included the Ikaros locus, predominantly by large interstitial deletion (of one or both copies) or deletion extending to the most centromeric marker examined; although, LOH in some tumors was by retention of two copies of chromosome 11 from a single parent. The frequency of LOH or deletions involving Ikaros was higher in both of the older groups than in the infant-irradiation group. In all but one tumor, LOH on chromosome 11 always included the Pten locus. Interestingly, LOH on chromosome 19 was predominantly via retention of two copies from a single parent, either along the length of the chromosome, or extending from near the Pten locus down to the most telomeric marker examined; although, smaller interstitial deletions (of one or both copies) centered on the Pten locus were also observed. In contrast to the Cdkn2a and Ikaros loci, the frequency of LOH at the Pten locus was higher in the infant-irradiation group than in the two older groups.

2.3. Expression and mutation of IKAROS and PTEN

We examined the expression and sequence of Ikaros and Pten transcripts by RT-PCR and measured protein levels by western blotting. Mutation frequencies of Ikaros (including inactivation of both alleles, a single dominant-negative mutation or unexplained lack of expression) were 33% (5/15), 31% (4/13) and 50% (5/10) in the infant-, adolescent-, and young adult–irradiation groups, respectively. Lack of Ikaros protein was observed in the young adult–irradiation group in particular. In contrast, the mutation frequencies of Pten (including inactivation of both alleles or unexplained lack of protein), were 60% (9/15), 38% (5/13) and 30% (3/10) in the infant-, adolescent-, and young adult–irradiation groups, respectively. There were no significant associations between the status of Ikaros and Pten across the tumors that would indicate either coincidence or exclusivity of the mutation events.

2.4. Aberrations in other genes

In contrast to Cdkn2a, Ikaros and Pten, the Trp53 mutation frequency in lymphomas from all three irradiated groups was low in accordance with previous reports showing a low frequency of Trp53/TP53 mutation in mouse T-cell lymphoma and human leukemia. Furthermore, frequency of site-specific copy-number alteration did not show any other significant differences depending on age at exposure among the three-irradiation groups. Activating deletions in Notch1, deletion of Bcl11b, and trisomy of chromosome 15 were frequent events in all three irradiation groups. Copy-number alteration at the various T cell receptor gene loci, indicative of V(DJ) recombination-induced rearrangement, was in keeping with the developing T-cell origin of the tumors.

3. Summary

The incidence of mouse T-cell lymphoma showed no difference depending on age-at-irradiation. Nevertheless, our findings demonstrate that while deletions on chromosomes 4 and 11 affecting the Cdkn2a and Ikaros loci are a prominent feature of young adult irradiation–induced T-cell lymphoma, tumors arising after infant irradiation suffer a second hit in the Pten gene by chromosome mis-segregation or recombination (Fig.3). This is the first report showing an influence of age-at-exposure on genomic alterations of tumor suppressor genes and their relative involvement in radiation-induced T-cell lymphoma. These data are important for considering the risks associated with childhood exposure to radiation and suggest that the mechanism of carcinogenesis can vary even where cancer incidence does not change.

There was no difference in incidence of T-cell lymphoma among three irradiation groups.

Fig.3 Scheme of contribution of Pten and Ikaros mutations to T-cell lymphomagenesis. While the incidence of T-cell lymphoma did not show significant difference between three groups, the causal genes and the status of mutation depended on age at irradiation.

References

Tetsuo Nakajima, Guillaume Vares, Bing Wang, Mitsuru Nenoi
E-mail: nakajima.tetsuo@qst.go.jp

Introduction
Diet habits or nutrient factors have been demonstrated to influence radiation effects [1, 2]. However, among diet habits, how alcohol drinking habits modify radiation effects remain unclear. Many types of alcohol beverages are consumed worldwide. Sake is a traditional alcoholic beverage in Japan that is gaining popularity worldwide. Although sake is reported to have beneficial health effects, it is not known whether the habit of drinking sake modulates health risks due to radiation exposure or other factors. The liver is the main organ involved in detoxification of harmful substances, including alcohol and it is susceptible to radiation damage. Alcohol is metabolized in the liver, and the resulting metabolic byproducts can impair liver function and cause tissue damage. For this reason, liver metabolites are useful indicators of health status. Here, the effects of chronic administration of sake on radiation-induced metabolic alterations in the livers of mice and metabolic markers in serum were evaluated [3].

Metabolome analysis on effects of sake & 15% ethanol solution on mouse livers
Sake (junmai-shu) was administered daily to female mice (C3H/He) for one month, and the mice were exposed to fractionated doses of X-rays (0.75 Gy/day) for the last four days of the sake administration period. For comparative analysis, another group of female mice were administered 15% (v/v) ethanol in water instead of sake, and these mice were exposed to fractionated doses of X-rays (0.75 Gy/day) for the last four days of the 15% ethanol administration period. Metabolites in the liver were analyzed by capillary electrophoresis-time-of-flight mass spectrometry (CE-TOFMS) one day after the last exposure to radiation. In the analyses, a total of 230 metabolites (87 anions and 143 cations) and 245 metabolites (81 anions and 164 cations) were identified in the livers of mice administered sake and 15% ethanol, respectively. Principal component analysis (PCA) was performed to reveal differences in the metabolite profiles of the four treatment groups, which consisted of the control, radiation, sake, and the combination of sake administration and radiation (Fig.1). In the PCA score plot, the group that received a combination of radiation and sake was clearly separated from the other three groups by the second principal component (PC2, 18.9% proportion; Fig.1). The metabolite profiles of mice chronically administered sake in combination with radiation showed marked changes. On the other hand, In the case of 15% ethanol administration, the group that received a combination of radiation and 15% ethanol was not clearly separated from the other groups along either PC1 or PC2 [3].

Using the correlation coefficients between the PC scores and variables for factor loading, we identified liver metabolites in irradiated mice were affected by sake administration. Metabolites that reached significant levels (p<0.01) in the evaluation of positive and negative correlations using the correlation coefficients were selected from the PC2 data from mice treated with a combination of radiation and sake. In the selected metabolites, seven metabolites (3-dephospho-CoA, GSH, nicotineamide, cysteine glutathione disulfide, GMP, UMP, and sedoheptulose 7-phosphate) were significantly modulated in the livers of mice treated with radiation and sake compared to the levels in the control, and sake and radiation alone-treated mice.

We have also demonstrated changes in several metabolites, including methionine and valine, were induced by radiation alone, but they were not detected in the livers of mice who received chronic administration of sake [3].

Fig.1 PCA of metabolic data for the combined effects of sake and radiation on mouse livers.
**Sake induces anti-oxidative activities in livers**

Among the seven selected metabolites that were significantly modulated in the livers of mice treated with radiation and sake, GSH is an important regulator of redox homeostasis and GSH/GSSG (glutathione disulfide) is considered to be the major redox couple that determines anti-oxidative capacity. GSSG is the oxidized form of GSH, and the GSH/GSSG ratio is often used as an indicator of the cellular redox state. Here, the levels of GSH and GSSG significantly increased and decreased, respectively, in the livers of mice treated with a combination of radiation and sake (Fig.2). The changes in these metabolites were not observed in mice administered 15% ethanol instead of sake (Fig.2), suggesting that glutathione metabolism is specifically influenced by the consumption of sake.

**Sake and radiation induce decrease in TG**

Changes in the serum levels of several metabolic biochemical markers that were accompanied by alterations in liver metabolism in the four treatment groups were also evaluated [3]. In this experiment, the amount of sake administered to mice seemed to be excessive because a significant increase of serum TG (triglycerides) in mice administered sake alone was observed compared to control mice (Fig.3). Although radiation alone induced a slight reduction of TG levels, the serum TG level in the treatment group that was administered sake was greatly reduced by radiation to the level of the control mice.

The observed reduction of TG by radiation in mice administered sake may be in part due to an induction of anti-oxidative responses, as indicated by the increase of GSH in the liver because the alcohol-induced accumulation of TG can reportedly be mitigated by a diet including foods that contain factors that promote anti-oxidative responses.

**Conclusions**

Chronic Japanese sake consumption induces specific metabolic alterations in the liver in response to irradiation. Although excess sake consumption may induce adverse effects on the liver, sake intake has the potential to promote anti-oxidative stress activities following radiation exposure in the liver. As many other aspects in the biological modulation of radiation effects by drinking sake have to be evaluated more, the findings presented here suggest that moderate sake consumption may promote anti-oxidative activity following exposure to stress such as radiation at least in the liver, thereby limiting the adverse effects typically associated with these stresses.

---

**Fig.2** Effects of sake or ethanol on radiation-induced changes of GSH and GSSG in mouse livers.

**Fig.3** Effects of sake on TG (triglycerides) in the serum of irradiated mice.

**References**


Highlight

Chronic restraint-induced stress seems to have little Impact on radiation hematopoietic toxicity in mice

Bing Wang, Kaoru Tanaka, Takanori Katsube, Yasuharu Ninomiya, Guillaume Vares, Tetsuo Nakajima, Mitsuru Nenoi
E-mail: wang.bing@qst.go.jp

Introduction
Both ionizing radiation (IR) and stresses cause detrimental effects on humans [1]. Besides possible health effects resulting directly from exposure to IR, a nuclear plant accident is a cause of social psychological stresses (PS). Using a mouse PS model, a recent study showed that chronic restraint-induced stresses (CRIS) attenuated Trp53 functions and increased carcinogenesis (predominantly lymphomas and sarcomas) susceptibility of Trp53 heterozygous (Trp53^{+/-}) animals to total-body γ-irradiation, having a big impact on the academic world and a sensational effect on the public, especially residents living in areas contaminated by radioactive materials from such an accident. It is important to investigate the possible modifying effects from CRIS on IR-induced health consequences in Trp53 wild type (Trp53^wt) animals. Prior to a carcinogenesis study, effects of total-body X-irradiation (TBXI) on the hematopoietic system under CRIS were investigated on hematological abnormality in the peripheral blood and residual damage in the bone marrow erythrocytes using a mouse PS model [2].

Materials and Methods
Four-week-old male Trp53^wt C57BL/6J mice were purchased from SLC, Inc., Japan. The mice were acclimatized to the laboratory conditions for 1 week as an adaptation period before use: they were maintained in a clean conventional animal facility under a 12-h light/12-h dark photoperiod. The mice were housed in autoclaved cages with sterilized wood chips, and allowed free access to acidified water (pH = 3.0 ± 0.2) and a standard laboratory chow MB-1 (Funabashi Farm Co., Japan). The mice at postnatal age 5 weeks were randomly assigned to 4 experimental groups, namely, the “control group (C-Gr)” receiving neither restraint nor TBXI, the “restraint group (R-Gr)” receiving only restraint, the “TBXI group (IR-Gr)” receiving only TBXI, and the “restraint and TBXI group ((R+IR)-Gr)” receiving both restraint and TBXI. For the mice in R-Gr and (R+IR)-Gr, the mouse restraint system (Flat Bottom Rodent Holder, RSTR541, Kent Scientific Co., USA) was used for chronic periodic restraint on a daily basis of 6 hours for 28 consecutive days. Individual mice were placed in the strainer and the restrained mice were maintained horizontally in their home cage during the 6-h restraint session (9:30 a.m. to 3:30 p.m.) daily, then the animals were released into the same cage and allowed to access food and water during the free session (3:30 p.m. to 9:30 a.m. the next day). The animals in C-Gr and IR-Gr received no restraint but they were kept from food and water from 9:30 a.m. to 3:30 p.m. each day. For the mice in IR-Gr and R+IR Gr, they were given an acute TBXI (4 Gy) on the 8th day. X-rays were generated with an X-ray machine (Pantak-320S, Shimadzu, Japan) operated at 200 kVp and 20 mA, using a 0.50-mm Al + 0.50-mm Cu filter. The dose rate was at 0.25 Gy/min. The body weight gain of the animals in each experiment group was recorded daily. At the end of the restraint regimen, the animals were euthanized. The peripheral blood hemogram was assessed and the bone marrow micronucleus test was carried out accordingly [3]. Bone marrow smears prepared from both femurs were processed for the enumeration of micronucleated polychromatic erythrocytes (MNPCes) and micronucleated normochromatic erythrocytes (MNNCes). The slides were coded to avoid any observer bias. The micronuclei were scored using a light microscope at a magnification of 1000x. At least 5000 cells per mouse were counted and the data for each experimental point were from at least 5 mice. All experimental protocols involving mice were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences (NIRS). The experiments were performed in strict accordance with the NIRS Guidelines for the Care and Use of Laboratory Animals. Statistical evaluation of the body weight data was done by 2-way ANOVA. For the other data Student’s t-test was used except for the micronucleus data where the χ² test was performed. Statistical significance was assigned to a value of P of <0.05.
Results

Significantly reduced body weight gain by CRIS appeared one day after onset of the restraint, which resulted in the lowest body weight on the 3rd day (Fig.1). After TBXI, significant reduction of body weight gain was observed on the following day in both IR-Gr and (R+IR)-Gr while no interaction (namely, neither synergistic nor antagonistic effect) between the restraint and TBXI was observed. The recovery of body weight gain appeared late in the animals that received the restraint (R-Gr and (R+IR)-Gr). In general, there was a statistically significant difference in the mean body weight between the groups that received the restraint and the groups that received no restraint 1 day after the onset of restraint regardless of the TBXI.

CRIS alone induced a marked decrease in red blood cell (RBC) and white blood cell (WBC) counts, while TBXI caused significant low counts of RBCs, WBCs and blood platelets, and low concentration of hemoglobin regardless of CRIS (Fig.2). CRIS alone did not show any significant effect on erythrocyte proliferation and on induction of micronucleated erythrocytes, while TBXI markedly inhibited erythrocyte proliferation and induced a significant increase in the incidences of micronucleated erythrocytes regardless of CRIS (Fig.3).

These findings suggest that CRIS does not have a significant impact on radiation-induced detrimental effects on the body weight gain and the hematopoietic system in Trp53 wt mice.

Discussion and Conclusion

Results obtained in the present study are consistent with the report on increased susceptibility induced by CRIS for Trp53 +/- mice to radiation carcinogenesis. It should be noticed that although the mice used were of the same strain, Trp53 wt animals were used in this work. It is known that chronic stresses-induced susceptibility to pathogens and toxicological assaults including IR on health is dependent on the genetics of the exposed organism. Based on these studies and the results obtained in the present work, it was suggested that CRIS would have little influence on sensitivity of Trp53 wt mice to radiation effects on the hematopoietic system, including the genotoxic effect. The possibility still could not be excluded that the methodology of the present work is not sensitive enough to detect the influence of CRIS on the genotoxic effect in this experimental system. To improve the sensitivity for detection of genomic damage, further study using the fluorescence in situ hybridization technique for detection of chromosome aberrations in splenic cells is in progress.

In summary, the present findings suggest that CRIS does not have a significant impact, neither synergistic nor antagonistic, to modify the radiation-induced detrimental effects on the hematopoietic system in young Trp53 wt mice under the experimental setup used here. For most people, especially those living in radioactively contaminated areas, the present work may partially allay their concern that stresses could increase the cancer susceptibility to radiation.

References

Development of the retrospective animal archive and the international collaboration

Shin Saigusa
E-mail: saigusa.shin@qst.go.jp

**Introduction**

It is well known that radiation health risk estimations for radiation protection are based on the epidemiological data from the LSS (Life Span Study) of atomic bomb survivors. However, these health effect data are the results of the single- and acute-exposure to relatively high-dose and high-dose rate radiation, and therefore, they require an extrapolation to low-dose and low-dose rate using a reduction factor to apply these values to the practical doses of radiation protection, i.e., over ten mSv order. Though there are no suitable epidemiological data at present, the reduction factor for this purpose is estimated by long-term animal experimental data and numerical model analysis. Therefore, the storage of animal exposure experimental data and the reposition of derivative biomaterials of both previous and present studies are valuable and important.

Archival activities may be categorized into two types, prospective archiving and retrospective archiving. In short, the former includes the archiving activity which is concurrently proceeding with the ongoing studies. Conversely, the latter includes the activity which is archiving the data and materials of terminated studies. The activities described in this report are mainly focusing on the retrospective type of archiving.

The retrospective type of archival and repository activities to digitalize and storage the data/materials of long-term animal experiments was started in the early 1990’s in institutes of both the US and Europe. These archival activities included the collection and digitalization of the primary data and information (detailed exposure protocols, animal data and pathological diagnosis, etc.) and storage of the experimental materials (paraffin embedded tumor blocks, derivative slides). Data and exposed materials have been correlating to each other by using a common data registry format.

Long term animal experiments have become considerably difficult to perform on a large-scale, because research grants have been reduced and the numbers of investigators, particularly radiation pathologists, have dropped as well. According to the national intellectual infrastructure development plan, NIRS has started to archive its research products as well as the measurement standards.

**Scientific background**

Differences between animal data and models adopted in research organizations are reflected on the resulted risk estimates, e.g., National Academy of Sciences – National Research Council estimated the DDREF (Dose and Dose Rate Effectiveness Factor) as 1.5 in its 2006 BEIR VII Report [1] and ICRP (International Commission on Radiological Protection) estimated it as 2 in its 2007 Recommendations [2].

In the early 1990’s, in order not to lose valuable information, U.S. and European scientists started an international collaborative project to collect data and store exposed biomaterials. This project, with the cooperation of Japan, has been followed up with a series of successive projects of the EU (e.g., ERA, ERA-PRO, and STORE shown in Fig.1 and the US (Janus Tissue Archive).

Accumulated archival data have been used for radiation risk reanalysis [3] or meta-analysis by compiling different data sets from previous studies and the exposed materials are being provided for checking by recent molecular analysis techniques.

**Development of international network**

To enhance the international collaboration, the 1st International Workshop on Sample/Tissue Archiving of Radiobiology (STAR2015) was organized as a satellite meeting of the International Congress on Radiation Research in Kyoto (ICRR 2015) on 24-25 May 2015 and NIRS co-chaired this meeting. The purpose of this workshop was to provide researchers with information about the structure of archive systems worldwide, technology for research analysis of archived materials, and the future possibility to organize an academic network and international research collaboration for sustainability of the archives as the property for the next generation. A total of 26 participants from 4 countries joined this workshop and 14 presentations were made. Table 1 shows the archives and databases introduced in this meeting. It was concluded that this workshop will be organized regularly every 4 years.
Research for Radiation Protection

Legacy materials to be archived

As a result of reorganization of NIRS, some of the legacy data and biomaterials of the long term animal experiments carried out for well over a decade are expected to be released from the storage management by the institute. Such data and materials are the results of the following studies:

1. Radiation-induced myeloid leukemia in C3H/He mice and the effect of prednisolone acetate on leukemogenesis;
2. Radiation-induced myeloid leukemia in C3H/He mice calorie restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice;
3. Radiation-induced myeloid leukemia in mice exposed to the low dose rate radiation; and
4. Radiation-induced tumor in mice exposed to fast neutrons, heavy particle beams and gamma rays.

It is planned that data and materials related to the above studies will be digitalized and redeposited as a part of the retrospective archive in the near future.

Conclusion

NIRS is now planning to take over several research departments of JAEA (Japan Atomic Energy Agency) and rebuild them into the new organization on 1 April 2016. This reorganization is expected to be accompanied by a wide range of restructuring in both administrative systems and research environments. Such reorganization of the institutions and the related transfer/retirement of the investigators will result in the disposal of the research deliverables. It is important to archive the legacy materials of the past studies systematically and reproduce them for the next generation.

References

